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## NITROGENOUS METABOLISM OF *PYRUS MALUS* L.

## III. THE PARTITION OF NITROGEN IN THE LEAVES, ONE AND TWO YEAR BRANCH GROWTH AND NON-BEARING SPURS THROUGHOUT A YEAR'S CYCLE*

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(WITH FOUR FIGURES)

### Introduction

OSBORNE and his colleagues (**23**) in discussing the status of plant chemistry state: "It is true that we know a multitude of products derived from plants, and we know much of the chemistry of these, but this knowledge consists mostly of isolated facts which contribute comparatively little to a knowledge of the chemical make-up of the plant as a whole." This situation is due to the fact that the largest contributions to our knowledge of the compounds formed by plants have emanated from investigators in the chemical laboratories of large pharmaceutical manufacturing establishments who have vigorously prosecuted research in this direction for the purpose chiefly of isolating products of interest and value in therapeutics. Such investigations are not concerned with the energetics or dynamics of the plant, *i. e.*, with the functions and fate of the compounds isolated, in their relation to the response of the plant to the internal conditions existing during successive stages of their life cycle, by whatever means these may have been produced.

The theories of LIEBIG, though shown later to be false, spurred the early agricultural chemists and physiologists to carry out a prodigious amount

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of work on the quantitative changes of the various elements—ions—absorbed by the plant throughout the principal vegetative periods. Much of this work is abortive because the methods then used do not conform to modern standards of accuracy. The rapid advances during the last two decades in the chemistry of proteins and the products of their hydrolysis necessitates the investigation of the function of nitrogen with the application of this newer knowledge.

### Scope and limitations of this investigation

The investigation of the dynamic systems produced by the large number of chemical reactions which have given rise to the various nitrogen compounds in the plant, and of the function of the individual groups in metabolism, is a laborious task involving much observational and analytical work. A quantitative measure of the changes undergone by the various nitrogen groups throughout a year's cycle is outlined in this paper. But what specific rôle is played by each in the metabolic cycle and in what manner their activity can be controlled are problems for further investigation. In the next paper it will be shown in what manner fertilizer application of an inorganic salt like $NaNO_3$ can modify the seasonal changes in some of the principal nitrogen fractions.

It is doubtful if, in the present state of our knowledge, all the data accumulated in these investigations can be interpreted. A great deal of information and insight, however, can be obtained with respect to the transformations taking place and also to the relationships of the various fractions to one another. The bearing of the results presented in this paper to practical horticulture has been presented in a previous report (**37**). It is of interest to note that no relationship could be discovered between either temperature or sunshine or rainfall or humidity and any of the nitrogen fractions.

The content of soluble nitrogenous constituents of the cells from aerial parts of *Pyrus malus* is relatively low (**35**); thus, only seven to ten per cent. (depending upon the season of the year) of the total nitrogen of the leaves is soluble in water. This is the case whether the soluble cell products are obtained by means of cytolytic agents or by direct extraction with water. In these investigations preference is given to the latter method (**35**). Since nothing was to be gained by any further investigation of the soluble cytoplasmic proteins (**36**), the next logical procedure appeared to be an examination of the metabolically active or reactive phases of the products of the nitrogen transformations. These are measured by quantitative determinations, during a year's cycle, of the fluctuations of the various groups of nitrogen fractions soluble in water. The relationships of these fractions to one another and to any external factor or factors have also been investigated.

### Catalysers in growth and reproduction

The classical investigations of Emmerling (**10**) had indicated the relationship of amino-acids and amides to the synthesis of proteins, but their rôle in growth had not been stressed until MacDougal and Spoehr and McGee postulated that amino-acids and amides were the principal fractions influencing the rate of growth by functioning as catalytic agents, either by increasing the hydration capacity of the pentosan-protein colloids, as suggested by the former (**21**), or by accelerating the rate of carbohydrate katabolism, as indicated by the latter (**33**).

Although the investigation *in vitro* (even in a crude and rough fashion) of the course of definite reaction types, with the hope of discovering analogies between them and the complicated processes in the cell, may furnish valuable clues, nevertheless the extension and generalization of dogmatic interpretations and conclusions from experiments like MacDougal's, carried out *in vitro* to processes *in vivo*, must be made with considerable reservation. Moreover, in Spoehr and McGee's experiments dealing with the effect of feeding glycocoll on the stimulation of the rate of respiration and carbohydrate consumption, the plants and also excised leaves were subjected to more complete shading than would occur under normal growth conditions; hence, the conclusions drawn are hardly applicable to the normal processes of the plant. Again, the behavior of nitrogenous compounds as catalytic agents is not peculiar to amino-acids, for Dakin (**9**) found that not only amino-acids but also peptone could be used to catalyse certain condensations at low temperatures, and Knoevenagel (cited by Dakin, *loc. cit.* p. 50) found that even organic bases (diethylamine, piperidine, aniline, and other bases) could be used to bring about a number of syntheses, at low temperatures, such as the union of aldehydes and peptones, and substances containing the $-CO-CH_2.CO-$ group. Since the other nitrogen fractions, *viz.*, ammonia, amides, peptides, basic compounds and also those unclassified nitrogen compounds that do not respond to the tests in any scheme thus far developed for partitioning the complex nitrogenous compounds of plants—the so-called "rest" N fraction—are shown in these experiments to take part in the nitrogen transformation during the vegetative cycle, the specific rôle of each fraction in relation to the synthesis and degradation of proteins, and, therefore, in the growth and reproduction processes must also be considered. The present investigations indicate that these "rest" N compounds may play as important a rôle as the amino-acids. The action of catalyzers is attributed to their capacity to exist in different states of oxidation. Nitrogen, copper, manganese and iron, for this reason, are especially active catalytic agents. Cook (**6**) has given evidence from which one may deduce that iron and copper are the

prime oxidation catalysts in cell metabolism. The stressing of the importance of amino-acids has had the unfortunate effect of leading some to believe that determination of these acids may indicate or be a measure of the vigor of growth; but this hypothesis is not borne out in the present investigations. It is possible, therefore, that the "rest" N compounds and possibly other fractions may play a rôle equally as important as amino-acids.

## Materials and methods

ELIMINATING VARIABLES.—Since the different parts of a fruit tree appear to behave as independent units (**1, 19**) and because variations of composition occur from the tip to the basal part of the shoot (**39**), the elimination of all controllable variables is essential. For this reason, in all experimental work involving analyses, fertilizers should be applied uniformly around the root system and care exercised to sample the tree uniformly. It is obvious, also, that if comparisons are to be made between two or more trees they should be of the same age and variety, growing in a homogenous soil. These principles were adhered to in these investigations.

DIFFICULTIES INHERENT TO ANALYTICAL INVESTIGATIONS IN PERENNIAL WOODY PLANTS.—HARVEY and MURNEEK (**14**) have discussed the analytical difficulties of experimental work with woody perennials such as fruit trees in comparison with herbaceous annuals like the tomato. Reference has been made to one of the disadvantages, *viz.*, the behavior of the different parts of fruit trees as independent units; another disadvantage lies in the relatively small percentage of active tissue present, in consequence of which special refinement in analytical methods is essential. Still another difficulty that is not encountered in herbaceous plants lies in the relative alteration in the proportions of wood to bark with age. The ratio of wood to bark varies from the apex to the base of the branch in the sense that it increases with the age of the growth. A determination of the *absolute* changes in magnitude of any group or constituent throughout the vegetative cycle is, therefore, precluded. However, relative fluctuations and especially the direction of these changes, as influenced by external factors, can be studied. The difficulty of the wood-to-bark ratio may, in part, be overcome by separating the bark from the wood and analyzing each separately; but even this method is not free from criticism, for during the interval required to separate the bark from the wood enzymatic changes can take place that might seriously affect the results. Analyses have been made both with and without separation in these investigations, with the result that no advantage is derived from such a separation. Moreover, the interpretation of results is not changed by the method of procedure in this respect, because, although the bark is higher than the wood in total nitro-

gen, it is lower in most of the water-soluble fractions; consequently, when the curves for amino-acids and amides, for example, are drawn for the wood and the bark separately throughout the cycle, the relative increase in mass of wood to bark is counterbalanced by the somewhat higher amounts of these fractions in the wood compared with the bark.

A Stayman Winesap tree, 15 years old, in the "off" bearing year, of known history and performance, growing in sod in the College Experimental Orchard, was used in this investigation. It received an application of 5 lbs. $NaNO_3$ on April 7, 1923. The desirability of restricting the work to one tree is obvious as a means of eliminating variability. Although a composite sample of a number of trees, provided they were of the same variety and age, if available, would give larger samples, thus facilitating analytical work, the possible advantages to be gained by this procedure would be counterbalanced by the disadvantages attendant upon the inability, in a preliminary investigation, to focus attention upon the behavior of one plant.

Samples of leaves, non-bearing spurs, and branches of three different ages (one year, two years, and three to seven years) were taken at eleven dates between June 18, 1923, and June 11, 1924. The sampling was done at times when the meteorological (rainfall and humidity) conditions had been favorable for three consecutive days preceding the taking of the samples. The diurnal variations which occur in the tissues of the apple tree were found to be small compared with the seasonal changes. They were avoided by always taking the samples at the same time of the day, in the early morning. Although a small decrease occurred in the total nitrogen at night, no changes could be observed in the total water-soluble products during this period.

## Fractionation methods

The method of extraction has already been described (**35**). Some investigators effect the extraction of nitrogenous constituents by means of boiling water. This method is, however, objectionable not only because a certain degree of peptonization of proteins may take place, but many nucleins are decomposed by boiling water with formation of hypoxanthine, etc. (**30**). Water at 40° C. was used for the extraction in this investigation.

Any method of separating the nitrogen fractions will necessarily be based upon the work of the army of investigators who have contributed to our knowledge of the analysis of proteins and their hydrolytic products. Among the contemporary workers who have sought to apply the nitrogen distribution methods to heterogeneous compounds of plant origin should be mentioned Chibnall, Williams, and the Illinois group

THE FRACTIONATION METHODS ADOPTED IN THESE INVESTIGATIONS

SCHEME I

A 500 cc. aliquot of the non-protein N filtrate (B) (*i.e.*, the filtrate from the colloidal ferric hydroxide precipitate) is treated according to (a). The remainder is hydrolyzed according to (b).

(a)
500 cc. is distilled *in vacuo* with $Mg(OH)_2$.

Ammonia (c) is determined in the distillate.

Residue (d) filtered from "melanin" N (e) and free (mono- and di-) amino N (f) is determined in the filtrate (D) after concentration *in vacuo*.

(b)
Remainder of filtrate (B) is hydrolyzed with 20 per cent. HCl and distilled with $Ca(OH)_2$.

Distillate (g) contains free ammonia (c) + free and combined amide N (j).

Residue (h) concentrated and filtered from $Ca(OH)_2$ "melanin" and humin N (k). Free and combined mono- and di-amino N (1) is determined in the filtrate (F) after concentration *in vacuo*.

(1)—(f) gives combined mono- and di-amino N.

SCHEME II

The *whole* of the non-protein N filtrate (B) is taken and hydrolyzed with 4 per cent. HCl.

Distillate (a) contains free ammonia and asparagine N.

Residue (b) is filtered from the $Ca(OH)_2$ "melanin" N and humin N (d).

Filtrate (c) treated after concentration with phospho-tungstic acid (H. P. W.).

Free mono-amino N is determined in the filtrate (d).

Residue (e) contains the basic N compounds.

(HAMILTON, GRINDLEY, and colleagues), who have contributed numerous papers in this field.

Two different methods of separation have been used by the writer as a basis for the characterization of the various non-protein fractions. These two methods are, for clarity, represented here diagrammatically.

The two schemes differ with respect to the rigor of the hydrolysis, resulting in a differentiation with respect to (*a*) the distribution of the mono-amino- and the di-amino-acids, and (*b*) of the substances determined as amide N. Most of the complex peptide linkings would be decomposed by the stronger hydrolysis with 20 per cent. HCl, so that whereas the amide N in fractionation scheme I includes the combined as well as the free $-CO-NH_2$ groups, the mild hydrolysis adopted in fractionation scheme II gives as amide N only asparagine and other similarly constituted acid amides, *i. e.*, those having the $-CO-NH_2$ group free. Again in scheme I the free amino (both mono- and di-) and also the combined amino N (both mono- and di-) are determined *separately,* whereas in scheme II compounds containing the combined di-amino N groups are precipitated with the bases. The latter fractionation method will, therefore, give only the free mono-amino N.

A group of not very clearly defined nitrogenous compounds, *viz.*, the so-called "humin" and "melanin" fractions are separated in both fractionation methods. The origin of the "humin" N from the hydrolysis of proteins has been studied by GORTNER *et al* (**11, 12, 13, 16**). The term "melanin" N is used by the writer to denote the compounds precipitated by $Ca(OH)_2$ or $Mg(OH)_2$. It represents the nitrogen belonging to the acid pigment-like bodies found in the sap of plants. MORROW and GORTNER (**22**) believe that this nitrogen may also be derived from the calcium salts of the purine and pyrimidine bases. This, however, is questionable, for the barium salts of the pyrimidines are quite soluble as are also some of the alkali earth salts of the purines like thiobromine sodium.

As already mentioned, there remains in both fractionation schemes a group of nitrogen compounds, usually designated as "rest" N or "other" N, which do not respond to the tests in any scheme thus far developed for determining the partition products. This residual N cannot represent identical nitrogen compounds in the two methods. Thus, in scheme I the "rest" N will represent the difference between the total non-protein N and the sum of the ammonia, free (mono- and di-) amino, combined (mono- and di-) amino, free and combined amide and also "humin" nitrogen; while in scheme II, based on the HAUSMANN (**15**) separation of the mono- and di-amino acids by phosphotungstic acid (H. P. W.), the "rest" N will represent the difference between the total non-protein N and the sum of the ammonia, free mono-amino N, asparagine N (amide N) (**28**).

basic N and "humin" N. These basic nitrogen compounds comprise the derivatives of trimethyl-amine, hygrine, adenine, choline, betame, stachydrine; the purine bases; and also the basic di-amino compounds like arginine, histidine, lysine and cystine.

The first fractionation scheme then determines both mono- and di-amino acids, but the bases and di-amino acids are removed by phosphotungstic acid in the second fractionation method, and which, accordingly, estimates only the mono-amino acids. It will be shown in a later paper that the "rest" N compounds of scheme II are higher throughout the cycle than those of scheme I. This indicates (*a*) that the peptide combinations present *in these filtrates* are not precipitated by phosphotungstic acid, though doubtless, as found by VICKERY (**43**), some polypeptides may be precipitated by phosphotungstic acid; or (*b*) that the combined mono- and di-amino N compounds (*i. e.*, compounds containing peptide linkages) are present in the non-protein fraction in much larger amounts than the basic nitrogen; or (*c*) that a portion of the "rest" N fraction has undergone decomposition during the more vigorous hydrolyses adopted in scheme I. Thus, CHIBNALL (**3**) found in his starvation experiments that hydrolysis with strong acids completely decomposed the "rest" N compounds, half their nitrogen appearing as ammonia N and about one-third as bases.

Without going into details respecting the nature of the substances precipitated by phosphotungstic acid, from the literature of the subject (**18, 24, 31**), it is certain that a variable product is obtained, depending to some extent on the concentration of the solution, and *even in the case of the decomposition products of pure proteins,* other decomposition products besides the purines and the di-amino acids, histidine, arginine, and lysine appear to be precipitated by phosphotungsic acid, according to conditions. Probably leucine and tyrosine may also be precipitated (**31**). KIESEL (**18**) under the conditions of precipitation used by him found both phenylalanine and aspartic acid in the phosphotungstic acid precipitate. These results are undoubtedly due to the working conditions. Since the phosphotungstates of some amino acids are relatively insoluble, as a precautionary measure, it is always desirable to dilute to a large volume before attempting separation of the bases (**42**).

The first fractionation scheme was adopted in the 1923-24 experiments which are described in this paper and the second scheme in the experiments to be described in a later paper. Although undoubtedly superior to the second scheme, from the standpoint of accuracy, the first method possesses the disadvantage of being time-consuming, owing to the additional separations required.

## Details of analytical procedure

Attention is called at the outset to the fact that nitric N was found only at one period—just as the buds are opening. This point has been described fully elsewhere (**38**).

(*a*) Ammonia N.—This includes the ammonia of ammonium salts and is usually described in the literature as "free" ammonia for the purpose of differentiating between this ammonia and that formed by the hydrolysis of amides. Inasmuch as the protein-iron gel (**35**) is more readily filtered if heated, and inasmuch as a slight decomposition of peptides occurs under certain not well-defined conditions, it is advisable to determine ammonia on a 200 cc. aliquot of the *original extract* denoted as (A), before removal of the water-soluble proteins. The determination is best carried out in the apparatus and by the method described by Van Slyke (**40**). It is only fair to state here, since it is not generally known, that the method for ammonia N is essentially that described by Longi (**20**), who distilled at a temperature of 38-40° C. with $Mg(OH)_2$. He noted that neither urea nor asparagine were decomposed under these conditions. The writer prefers solid CaO (**26**) to MgO for the reason described later.

(*b*) Free mono- and di-amino N.—The removal of the "free" ammonia must be first carried out as described under (*a*) *from a 500 cc. aliquot of the filtrate* previously freed from soluble proteins in the manner already described (**35**). This filtrate freed from proteins is denoted as (B.) CaO is now better substituted for MgO. It is advantageous to use only a very slight excess of *solid* CaO, the exact amount being determined by a preliminary titration (**26**). With this precaution little or no frothing occurs in the Van Slyke apparatus during the determination of amino N. Pure CaO is recommended in preference to MgO because in the experience of the writer the former occludes or absorbs more of the pigments to which most of the frothing can be attributed. No success was obtained with secondary octyl (caprylic) alcohol (even twice redistilled, B. P. 131° C.) which has been recommended for the purpose of reducing the surface tension and, consequently, the frothing (**41**). The blanks from this reagent, due presumably to the evolution of some gas that is not absorbed by the alkaline permanganate mixture, were found too high to justify its use in the determination of the relatively small amount of amino N measured in these experiments.

A small and somewhat variable quantity of *nitrogenous matter* will always be *precipitated,* occluded or absorbed by the CaO. This is "melanin" N and is removed by filtration and washed with hot water; the filtrate, which is denoted as (C), is acidified with acetic acid and afterwards con-

centrated under a pressure of 15 mm. to about 15 cc. using a CLAISSEN flask (**40**). In this concentrated solution a further precipitate containing Si, Al, Ca, Na and K salts usually separates out. This precipitate is removed by filtration and the filtrate, which is denoted as (D), made up to a definite volume prior to its introduction into the VAN SLYKE microapparatus (**40, 41**).

The writer has confirmed SPOEHR and MCGEE's observations (**32**) with respect to the alleged interference of carbohydrates in the nitrous acid method (cited by SPOEHR and MCGEE, *loc. cit.* p. 34, footnote). The determination of the amino N in aspartic acid was not altered by the presence of glucose or fructose.

(*c*) FREE AND COMBINED AMIDE N.—A definite volume of concentrated HCl sufficient to give a 20 per cent. solution is now added to the remainder of the original filtrate (B) containing the non-protein nitrogenous constituents, *i.e.*, to the extract remaining after the removal of the aliquots for the determination of free and combined amino N as under (*b*) above. The mixture is then hydrolyzed for twelve hours with the usual precautions; after the removal of the HCl *in vacuo* (**40**) the residue is taken up in water and distilled *in vacuo* with a slight excess of solid CaO. A titration of the distillate gives both the "free" ammonia N and the "free and combined" amide N. The difference between the titration values for ammonia obtained as described under (*a*) and (*c*) gives the "free and combined" amide N. The solution still remaining in the flask is denoted as (E).

(*d*) COMBINED MONO- AND DI-AMINO N.—The solution (E) left in the flask from the determination of the free and combined amide N (*c*) is filtered from the CaO "melanin" nitrogenous material, washed, and the combined filtrates and washings (F) acidified with acetic acid and concentrated *in vacuo* to a volume of about 15 cc. A determination of the amino N in this gives the "free" and also the "combined" amino N. The latter, which is indicative of the complexity of the peptide linkings, is determined by the difference in the values obtained in this determination and that given under (*b*).

## Experimental results

The results of the analyses by the foregoing methods are given in the following tables. Table I gives the percentages of imbibitional and total water in branches of different ages, and in the short spurs and leaves. Table II shows the water-soluble nitrogenous constituents of the leaves, branches of different ages, and short spurs. The partition of the non-protein fraction is shown for various collecting dates in percentages of the green weight and dry weight.

## TABLE I

THE PERCENTAGES OF IMBIBITIONAL AND TOTAL WATER

ONE-YEAR BRANCH GROWTH

| SERIES NO. | COLLECTING DATE | FRESH WEIGHT | DRY WEIGHT | IMBIBITIONAL WATER | TOTAL WATER |
|---|---|---|---|---|---|
| | | gm. | gm. | Per cent. | Per cent. |
| 1 | June 18, 1923 | 55.0 | 26.3 | 52.1 | 57.6 |
| 7a | July 16, 1923 | 102.0 | 50.7 | 50.3 | 55.3 |
| 15 | August 17, 1923 | 41.8 | 20.5 | 51.0 | 56.5 |
| 27 | September 17, 1923 | 129.0 | 60.0 | 53.5 | 56.7 |
| 34 | October 15, 1923 | 104.0 | 52.5 | 50.0 | 54.7 |
| 46 | November 20, 1923 | 125.0 | 66.2 | 47.0 | 51.8 |
| 56 | April 12, 1924 | 120.0 | 62.0 | 48.3 | 54.0 |
| 64 | April 21, 1924 | 80.0 | 40.0 | 50.0 | 54.0 |
| 80 | April 28, 1924 | 69.0 | 34.3 | 50.7 | 54.0 |
| 98 | May 13, 1924 | 90.0 | 40.0 | 55.6 | 57.9 |
| 118 | May 22, 1924 | 57.0 | 23.0 | 59.6 | 61.0 |
| 143 | June 11, 1924 | 56.0 | 23.8 | 57.5 | 58.0 |

TWO-YEAR BRANCH GROWTH

| SERIES NO. | COLLECTING DATE | FRESH WEIGHT | DRY WEIGHT | IMBIBITIONAL WATER | TOTAL WATER |
|---|---|---|---|---|---|
| | | gm. | gm. | Per cent. | Per cent. |
| 03 | June 18, 1923 | 75.0 | 35.4 | 52.8 | 54.2 |
| 7 | July 16, 1923 | 150.0 | 75.0 | 50.0 | 54.0 |
| 19 | August 17, 1923 | 69.5 | 33.8 | 52.0 | 54.5 |
| 31 | September 17, 1923 | 167.0 | 87.0 | 47.9 | 53.7 |
| 38 | October 15, 1923 | 155.0 | 85.0 | 45.2 | 50.7 |
| 47 | November 20, 1923 | 161.0 | 87.0 | 44.7 | 49.8 |
| 57 | April 12, 1924 | 65.0 | 35.1 | 46.0 | 48.0 |
| 65 | April 21, 1924 | 82.0 | 45.0 | 45.1 | 51.1 |
| 97 | April 28, 1924 | 125.0 | 67.0 | 46.4 | 52.4 |
| 119 | May 22, 1924 | 132.0 | 65.0 | 50.8 | 54.8 |
| 147 | June 11, 1924 | 140.0 | 70.0 | 50.0 | 53.8 |

OLDER GROWTH

| SERIES NO. | COLLECTING DATE | FRESH WEIGHT | DRY WEIGHT | IMBIBITIONAL WATER | TOTAL WATER |
|---|---|---|---|---|---|
| | | gm. | gm. | Per cent. | Per cent. |
| 4 | June 18, 1923 | 210.0 | 110.4 | 47.4 | 52.3 |
| 8a | July 16, 1923 | 230.0 | 118.4 | 48.4 | 52.3 |
| 14a | August 17, 1923 | 199.1 | 101.0 | 49.3 | 51.6 |
| 32 | September 17, 1923 | 174.0 | 98.0 | 48.0 | 50.2 |
| 39 | October 15, 1923 | 632.0 | 297.0 | 47.0 | 50.1 |
| 49 | November 20, 1923 | 455.0 | 244.0 | 47.0 | 50.2 |
| 58 | April 12, 1924 | 367.0 | 202.2 | 44.9 | 50.6 |
| 66 | April 21, 1924 | 165.0 | 89.0 | 46.1 | 49.5 |
| 82 | April 28, 1924 | 239.0 | 133.0 | 44.3 | 51.7 |
| 99 | May 13, 1924 | 196.0 | 100.0 | 48.9 | 53.2 |
| 121 | May 22, 1924 | 183.0 | 96.0 | 47.5 | 53.1 |
| 148 } 148a } | June 11, 1924 | { 419.0 { 127.0 | { 236.0 { 58.0 | { 43.7 { 54.3 | 52.4 |

SHORT SPURS

| SERIES NO. | COLLECTING DATE | FRESH WEIGHT | DRY WEIGHT | IMBIBITIONAL WATER | TOTAL WATER |
|---|---|---|---|---|---|
| | | gm. | gm. | Per cent. | Per cent. |
| 5 | June 18, 1923 | 31.0 | 15.1 | 51.3 | 57.0 |
| 7 | July 16, 1923 | 35.0 | 16.8 | 52.0 | 55.3 |
| 17 | August 17, 1923 | 26.5 | 11.4 | 47.0 | 53.0 |
| 29 | September 17, 1923 | 37.0 | 20.2 | 47.5 | 51.2 |
| 37 | October 15, 1923 | 121.0 | 65.2 | 46.3 | 51.3 |
| 48 | November 20, 1923 | 72.0 | 31.5 | 44.0 | 48.8 |
| 63 | April 12, 1924 | 58.0 | 30.0 | 48.3 | 54.0 |
| 69 | April 21, 1924 | 54.0 | 26.5 | 51.9 | 57.5 |
| 83 | April 28, 1924 | 38.0 | 19.0 | 50.0 | 58.7 |
| 96 | May 13, 1924 | 15.0 | 8.0 | 46.7 | 50.8 |
| 122 | May 22, 1924 | 22.0 | 9.0 | 49.1 | lost |
| 149 } 150 } | June 11, 1924 | { 2.5 { 5.5 | { 1.9 { 2.7 | { 24.0 { 50.9 | 46.0 |

LEAVES

| SERIES NO. | COLLECTING DATE | FRESH WEIGHT | DRY WEIGHT | IMBIBITIONAL WATER | TOTAL WATER |
|---|---|---|---|---|---|
| | | gm. | gm. | Per cent. | Per cent. |
| 01, 001 | June 18, 1923 | 38.0 | 17.2 | 54.8 | 57.0 |
| 6, 6a | July 16, 1923 | 242.0 | 111.2 | 54.1 | 63.0 |
| 18, 20 | August 17, 1923 | 127.0 | 51.8 | 59.8 | 65.5 |
| 28, 30 | September 17, 1923 | 406.0 | 185.1 | 52.4 | 59.0 |
| 35, 36 | October 15, 1923 | 516.0 | 245.0 | 52.9 | 60.5 |
| 50 | November 20, 1923 | 95.0 | 48.4 | 49.1 | 55.2 |
| 100 | May 13, 1924 | 47.0 | 13.0 | 72.3 | 75.0 |
| 119a, 123 | May 22, 1924 | 72.0 | lost | lost | lost |
| 144 | June 11, 1924 | 50.2 | 18.6 | 62.5 | 66.5 |
| 205 | August 17, 1924 | 150.0 | 58.2 | 61.2 | 65.0 |

### ACCURACY AND LIMITS OF ERROR

The errors of sampling could not be determined on account of the necessity for conserving the material, but the determination of the accuracy and limits of error of the analytical data has been made by expressing, as a percentage of the mean, the differences obtained in certain duplicate analyses. These are given in table III.

The total N shows a maximum difference of 0.58 per cent., the water-soluble N of 1.50 per cent., and the "free" mono- and di-amino N of 5.20 per cent. The experimental error in the "free" amino N is higher than that of the total nitrogen and total water-soluble N, because of the smaller amount of nitrogen determined.

Since the amide N is determined from the difference between the total $NH_3$ before and after hydrolysis, this determination will carry the errors of the ammonia determination also. It shows a maximum difference of 7.15 per cent. From the foregoing it is clear that all fluctuations in the nitrogen fractions can be regarded as significant.

## Discussion and conclusions

### (a) TOTAL NITROGEN

In general, the total nitrogen of the leaves (fig. 1) follows the course found for this species by other investigators (**2, 17**); these changes will, therefore, not be elaborated upon here, except to point out that the change

## TABLE II

AMOUNT OF WATER-SOLUBLE NITROGENOUS SUBSTANCES PRESENT IN THE LEAVES, 1923 BRANCH GROWTH, 1922 BRANCH GROWTH, SHORT SPURS, AND THE PARTITION OF THE NON-PROTEIN PORTION EXPRESSED AS PERCENTAGES OF FRESH AND DRY WEIGHTS, RESPECTIVELY

LEAVES

| SERIES NO. | COLLECTING DATE | TOTAL N | PROTEIN N | TOTAL WATER-SOLUBLE N | TOTAL NON-PROTEIN N | $NH_3$ N | HUMIN N | FREE AMINO N | COMBINED AMINO N | AMIDE N | RESIDUAL N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | As percentages of fresh weight of material | | | | | | | |
| 4A | June 18, 1923 | 0.719 | 0.0047 | 0.0606 | 0.0559 | 0.0013 | 0.0112 | 0.0162 | 0.0127 | 0.0094 | 0.0051 |
| 6 | July 16, 1923 | 0.691 | 0.0030 | 0.0510 | 0.0482 | 0.0017 | 0.0087 | 0.0104 | 0.0126 | 0.0081 | 0.0066 |
| 20 | August 17, 1923 | 0.641 | 0.0027 | 0.0430 | 0.0403 | 0.0024 | 0.0091 | 0.0070 | 0.0119 | 0.0024 | 0.0075 |
| 28 | September 17, 1923 | 0.715 | 0.0038 | 0.0570 | 0.0532 | 0.0059 | 0.0119 | 0.0156 | 0.0176 | 0.0016 | 0.0006 |
| 35 | October 15, 1923 | 0.646 | 0.0019 | 0.0422 | 0.0405 | None | 0.0022 | 0.0120 | 0.0135 | 0.0086 | 0.0040 |
| 50 | November 20, 1923 | 0.585 | 0.0009 | 0.0280 | 0.0271 | None | 0.0013 | 0.0034 | 0.0027 | 0.0106 | 0.0091 |
| 100 | May 13, 1924 | 0.877 | 0.0110 | 0.0820 | 0.0710 | ..... | ..... | ..... | ..... | ..... | ..... |
| 144 | June 11, 1924 | 0.671 | 0.0060 | 0.0410 | 0.0350 | ..... | ..... | ..... | ..... | ..... | ..... |
| | | | | As percentages of dry weight of material | | | | | | | |
| 4A | June 18, 1923 | 1.656 | 0.0110 | 0.1410 | 0.1300 | 0.0032 | 0.0261 | 0.0376 | 0.0300 | 0.0219 | 0.0120 |
| 6 | July 16, 1923 | 1.869 | 0.0100 | 0.1370 | 0.1270 | 0.0046 | 0.0270 | 0.0281 | 0.0345 | 0.0177 | 0.0180 |
| 20 | August 17, 1923 | 1.860 | 0.0076 | 0.1227 | 0.1151 | 0.0070 | 0.0250 | 0.0200 | 0.0341 | 0.0070 | 0.0220 |
| 28 | September 17, 1923 | 1.746 | 0.0095 | 0.1425 | 0.1350 | 0.0147 | 0.0298 | 0.0390 | 0.0440 | 0.0044 | 0.0010 |
| 35 | October 15, 1923 | 1.617 | 0.0048 | 0.1055 | 0.1013 | None | 0.0055 | 0.0300 | 0.0330 | 0.0215 | 0.0103 |
| 50 | November 20, 1923 | 1.570 | 0.0020 | 0.0622 | 0.0600 | None | 0.0028 | 0.0075 | 0.0060 | 0.0239 | 0.0200 |
| 100 | May 13, 1924 | 3.512 | 0.0440 | 0.3280 | 0.2840 | ..... | ..... | ..... | ..... | ..... | ..... |
| 144 | June 11, 1924 | 2.000 | 0.0530 | 0.1206 | 0.0676 | ..... | ..... | ..... | ..... | ..... | ..... |

## TABLE II—(*Continued*)

AMOUNT OF WATER-SOLUBLE NITROGENOUS SUBSTANCES PRESENT IN THE LEAVES, 1923 BRANCH GROWTH, 1922 BRANCH GROWTH, SHORT SPURS, AND THE PARTITION OF THE NON-PROTEIN PORTION EXPRESSED AS PERCENTAGES OF FRESH AND DRY WEIGHTS, RESPECTIVELY

1923 BRANCH GROWTH

| SERIES NO. | COLLECTING DATE | TOTAL N | PROTEIN N | TOTAL WATER-SOLUBLE N | TOTAL NON-PROTEIN N | $NH_3$ N | HUMIN N | FREE AMINO N | COMBINED AMINO N | AMIDE N | RESIDUAL N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | As percentages of fresh weight of material | | | | | | | |
| 1 | June 18, 1923 | 0.269 | 0.0086 | 0.0507 | 0.0421 | 0.0012 | 0.0130 | 0.0125 | None | 0.0077 | 0.0077 |
| 5 | July 16, 1923 | 0.324 | 0.0248 | 0.0739 | 0.0490 | 0.0014 | 0.0246 | 0.0175 | None | 0.0033 | 0.0022 |
| 15 | August 17, 1923 | 0.288 | 0.0119 | 0.0717 | 0.0602 | 0.0092 | 0.0098 | 0.0185 | 0.0016 | 0.0057 | 0.0114 |
| 27 | September 17, 1923 | 0.233 | 0.0025 | 0.0246 | 0.0221 | 0.0007 | 0.0051 | 0.0050 | Trace | 0.0052 | 0.0060 |
| 34 | October 15, 1923 | 0.297 | 0.0066 | 0.0337 | 0.0271 | 0.0011 | 0.0039 | 0.0055 | 0.0057 | 0.0101 | 0.0008 |
| 46 | November 20, 1923 | 0.328 | 0.0079 | 0.0451 | 0.0372 | 0.0020 | 0.0079 | 0.0079 | None | 0.0060 | 0.0134 |
| 56 | April 12, 1924 | 0.272 | 0.0091 | 0.0268 | 0.0177 | None | 0.0023 | 0.0039 | None | 0.0088 | 0.0025 |
| 64 | April 21, 1924 | 0.305 | 0.0142 | 0.0414 | 0.0262 | None | 0.0081 | 0.0105 | None | 0.0048 | 0.0012 |
| 80 | April 28, 1924 | 0.304 | 0.0121 | 0.0576 | 0.0483 | 0.0031 | 0.0184 | 0.0114 | 0.0061 | 0.0054 | 0.0037 |
| 98 | May 13, 1924 | 0.244 | 0.0071 | 0.0385 | 0.0280 | 0.0016 | 0.0026 | 0.0088 | ..... | 0.0075 | 0.0075 |
| 118 | May 22, 1924 | 0.238 | 0.0027 | 0.0230 | 0.0203 | 0.0013 | 0.0023 | 0.0072 | 0.0013 | 0.0068 | 0.0013 |
| 144 } 144A } | June 11, 1924 | 0.249 | 0.0056 | 0.0302 | 0.0245 | 0.0009 | 0.0047 | 0.0099 | 0.0024 | 0.0052 | 0.0015 |
| | | | | As percentages of dry weight of material | | | | | | | |
| 1 | June 18, 1923 | 0.372 | 0.0200 | 0.1180 | 0.0980 | 0.0028 | 0.0302 | 0.0290 | None | 0.0180 | 0.0180 |
| 5 | July 16, 1923 | 0.326 | 0.0552 | 0.1642 | 0.1090 | 0.0031 | 0.0546 | 0.0390 | ..... | 0.0073 | 0.0050 |
| 15 | August 17, 1923 | 0.352 | 0.0261 | 0.1630 | 0.1369 | 0.0209 | 0.0223 | 0.0420 | 0.0037 | 0.0210 | 0.0260 |
| 27 | September 17, 1923 | 0.367 | 0.0060 | 0.0600 | 0.0540 | 0.0018 | 0.0124 | 0.0121 | Trace | 0.0130 | 0.0146 |
| 34 | October 15, 1923 | 0.381 | 0.0146 | 0.0750 | 0.0604 | 0.0024 | 0.0086 | 0.0123 | 0.0126 | 0.0225 | 0.0017 |
| 46 | November 20, 1923 | 0.467 | 0.0165 | 0.0939 | 0.0774 | 0.0041 | 0.0164 | 0.0165 | None | 0.0124 | 0.0280 |
| 56 | April 12, 1924 | 0.419 | 0.0198 | 0.0582 | 0.0384 | None | 0.0050 | 0.0088 | None | 0.0191 | 0.0054 |
| 64 | April 21, 1924 | 0.380 | 0.0332 | 0.0900 | 0.0568 | None | 0.0177 | 0.0206 | None | 0.0105 | 0.0080 |
| 80 | April 28, 1924 | 0.347 | 0.0262 | 0.1252 | 0.1050 | 0.0068 | 0.0400 | 0.0248 | 0.0132 | 0.0117 | 0.0084 |
| 98 | May 13, 1924 | 0.315 | 0.0239 | 0.0874 | 0.0637 | 0.0037 | 0.0060 | 0.0200 | ..... | 0.0170 | 0.0170 |
| 118 | May 22, 1924 | 0.340 | 0.0060 | 0.0512 | 0.0452 | 0.0028 | 0.0050 | 0.0160 | 0.0030 | 0.0150 | 0.0029 |
| 144 } 144A } | June 11, 1924 | 0.398 | 0.0120 | 0.0642 | 0.0522 | 0.0020 | 0.0100 | 0.0210 | 0.0050 | 0.0110 | 0.0032 |

## TABLE II—(*Continued*)

AMOUNT OF WATER-SOLUBLE NITROGENOUS SUBSTANCES PRESENT IN THE LEAVES, 1923 BRANCH GROWTH, 1922 BRANCH GROWTH, SHORT SPURS, AND THE PARTITION OF THE NON-PROTEIN PORTION EXPRESSED AS PERCENTAGES OF FRESH AND DRY WEIGHTS, RESPECTIVELY

1922 BRANCH GROWTH

| SERIES NO. | COLLECTING DATE | TOTAL N | PROTEIN N | TOTAL WATER-SOLUBLE N | TOTAL NON-PROTEIN N | $NH_3$ N | HUMIN N | FREE AMINO N | COMBINED AMINO N | AMIDE N | RESIDUAL N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | As percentages of fresh weight of material | | | | | | | |
| 03 | June 18, 1923 | 0.230 | 0.0110 | 0.0321 | 0.0211 | 0.0009 | 0.0055 | 0.0059 | None | 0.0055 | 0.0033 |
| 8 | July 16, 1923 | 0.293 | 0.0179 | 0.0621 | 0.0442 | 0.0014 | 0.0224 | 0.0087 | 0.0067 | 0.0030 | 0.0020 |
| 19 | August 17, 1923 | 0.221 | 0.0139 | 0.0389 | 0.0249 | 0.0040 | 0.0019 | 0.0049 | 0.0045 | 0.0046 | 0.0050 |
| 31 | September 17, 1923 | 0.211 | 0.0047 | 0.0162 | 0.0113 | None | 0.0022 | 0.0025 | Trace | 0.0051 | 0.0014 |
| 38 | October 15, 1923 | 0.240 | 0.0089 | 0.0261 | 0.0172 | 0.0012 | 0.0027 | 0.0054 | 0.0012 | 0.0089 | 0.0035 |
| 47 | November 20, 1923 | 0.273 | 0.0131 | 0.0466 | 0.0335 | None | 0.0035 | 0.0080 | 0.0034 | 0.0060 | 0.0075 |
| | | | | As percentages of dry weight of material | | | | | | | |
| 03 | June 18, 1923 | 0.719 | 0.0240 | 0.0700 | 0.0460 | 0.0020 | 0.0120 | 0.0130 | None | 0.0120 | 0.0070 |
| 8 | July 16, 1923 | 0.691 | 0.0390 | 0.1350 | 0.0960 | 0.0030 | 0.0488 | 0.0190 | 0.0146 | 0.0066 | 0.0040 |
| 19 | August 17, 1923 | 0.641 | 0.0300 | 0.0836 | 0.0536 | 0.0086 | 0.0042 | 0.0106 | 0.0096 | 0.0100 | 0.0106 |
| 31 | September 17, 1923 | 0.715 | 0.0100 | 0.0350 | 0.0253 | None | 0.0048 | 0.0065 | Trace | 0.0110 | 0.0030 |
| 38 | October 15, 1923 | 0.646 | 0.0180 | 0.0530 | 0.0350 | 0.0024 | 0.0055 | 0.0110 | 0.0025 | 0.0180 | 0.0070 |
| 47 | November 20, 1923 | 0.585 | 0.0260 | 0.0928 | 0.0668 | None | 0.0070 | 0.0160 | 0.0068 | 0.0120 | 0.0150 |

## TABLE II—(*Concluded*)

AMOUNT OF WATER-SOLUBLE NITROGENOUS SUBSTANCES PRESENT IN THE LEAVES, 1923 BRANCH GROWTH, 1922 BRANCH GROWTH, SHORT SPURS, AND THE PARTITION OF THE NON-PROTEIN PORTION EXPRESSED AS PERCENTAGES OF FRESH AND DRY WEIGHTS, RESPECTIVELY

SHORT SPURS

| SERIES NO. | COLLECTING DATE | TOTAL N | PROTEIN N | TOTAL WATER-SOLUBLE N | TOTAL NON-PROTEIN N | $NH_3$ N | HUMIN N | FREE AMINO N | COMBINED AMINO N | AMIDE N | RESIDUAL N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | As percentages of fresh weight of material | | | | | | | |
| 2 | June 18, 1923 | 0.372 | 0.0140 | 0.0355 | 0.0215 | 0.0013 | 0.0032 | 0.0047 | None | 0.0086 | 0.0037 |
| 7 | July 16, 1923 | 0.326 | 0.0226 | 0.0502 | 0.0274 | 0.0019 | 0.0033 | 0.0104 | None | 0.0108 | 0.0009 |
| 17 | August 17, 1923 | 0.352 | 0.0111 | 0.0959 | 0.0848 | 0.0230 | 0.0030 | 0.0199 | 0.0290 | 0.0029 | 0.0066 |
| 29 | September 17, 1923 | 0.367 | 0.0098 | 0.0335 | 0.0237 | 0.0029 | 0.0036 | 0.0079 | None | None | 0.0093 |
| 36 | October 15, 1923 | 0.381 | 0.0102 | 0.0510 | 0.0407 | 0.0041 | 0.0104 | 0.0147 | None | 0.0080 | 0.0034 |
| 48 | November 20, 1923 | 0.467 | 0.0051 | 0.0446 | 0.0394 | None | 0.0102 | 0.0107 | 0.0037 | 0.0012 | 0.0134 |
| | | | | As percentages of dry weight of material | | | | | | | |
| 2 | June 18, 1923 | 0.844 | 0.0325 | 0.0825 | 0.0500 | 0.0030 | 0.0073 | 0.0110 | None | 0.0200 | 0.0087 |
| 7 | July 16, 1923 | 0.726 | 0.0505 | 0.1115 | 0.0610 | 0.0042 | 0.0077 | 0.0231 | None | 0.0240 | 0.0020 |
| 17 | August 17, 1923 | 0.752 | 0.0236 | 0.2040 | 0.1804 | 0.0489 | 0.0063 | 0.0423 | 0.0618 | 0.0061 | 0.0140 |
| 29 | September 17, 1923 | 0.749 | 0.0200 | 0.0684 | 0.0484 | 0.0060 | 0.0073 | 0.0161 | None | None | 0.0190 |
| 36 | October 15, 1923 | 0.780 | 0.0209 | 0.1040 | 0.0831 | 0.0084 | 0.0213 | 0.0300 | None | 0.0164 | 0.0070 |
| 48 | November 20, 1923 | 0.916 | 0.0099 | 0.0857 | 0.0758 | None | 0.0197 | 0.0207 | 0.0072 | 0.0024 | 0.0258 |

TABLE III

DIFFERENCES OBTAINED IN CERTAIN DUPLICATE DETERMINATIONS AS PERCENTAGE OF THE MEAN

| SERIES NO. | TOTAL N | TOTAL WATER-SOLUBLE N | MONO- AND DI-AMINO N | AMIDE N |
|---|---|---|---|---|
| 6 | 0.58 | 1.20 | 5.20 | 6.00 |
| 15 | 0.44 | 1.40 | 3.40 | 3.10 |
| 19 | 0.50 | 0.35 | 1.80 | 2.00 |
| 28 | 0.32 | 0.00 | 4.00 | 7.15 |
| 34 | 0.00 | 0.50 | 0.25 | 2.30 |
| 48 | 0.18 | 1.50 | 3.40 | 0.80 |
| 98 | 0.32 | 0.75 | 3.80 | 1.50 |

in direction observed in September has occurred at approximately the same period every year the experiments were in progress. It is probably due to the diminished requirement of the branch growths for the nitrogen synthesized by the leaves.

THE EVACUATION OF NITROGEN FROM THE LEAVES IN THE FALL.—This so-called "Rückwanderung" or autumnal evacuation of nitrogen from the leaves to the branches in the fall has been asserted and as vigorously denied (**8**). Much of the difficulty in interpretation, however, can be attributed to the fact that in many instances attempts have been made to draw conclusions from analysis of the leaves only, omitting analysis of the perennial parts. Both COMBES (**4, 5**) and RIPPEL (**27**) have recently reported an extensive study of this phenomenon in young oak and beech trees. According to the former, the migration of nitrogenous substances commences as soon as the leaves turn yellow, slowly at first and then more rapidly. This is not in agreement with the results of RIPPEL (*loc. cit.*) nor of the writer; these latter clearly indicate that the evacuation of nitrogen in the leaves is not initiated suddenly at the onset of yellowing but much earlier. If the decrease commenced just as soon as yellowing occurred it might be argued that, since the nitrogen present in the leaves depends upon the difference between the amount of protein synthesized and that utilized in growth and respiration, and that, inasmuch as protein synthesis decreases in the autumn owing to the degeneration of the chloroplasts, this affords an explanation of the migration provided removal goes on at the normal rate. However, since there is sufficient evidence (*loc. cit.*) to indicate that the decrease in nitrogen may occur before degeneration of the chloroplasts occurs, this migration cannot be due to the same causes as that which occurs normally during the life of the tree, but must be distinct from the normal process. Admittedly, it is

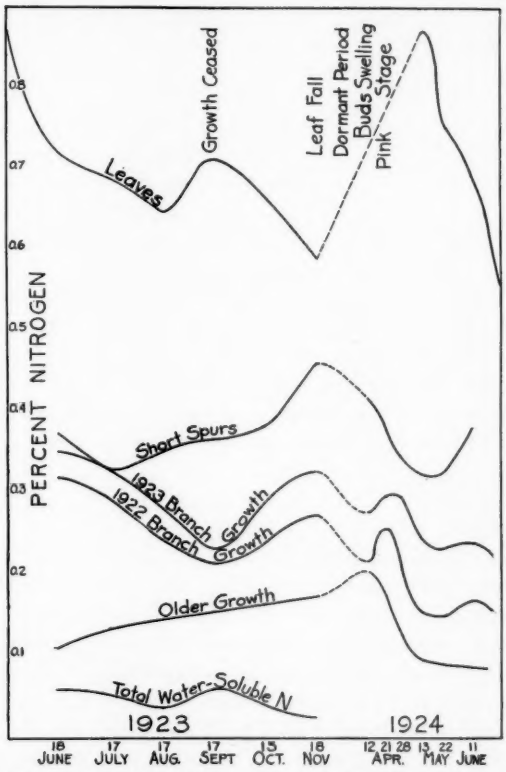


FIG. 1. Total nitrogen of the leaves, short non-bearing spurs, one-year, two-year and older branch growth as percentages of the fresh weight. June, 1923, to June, 1924.

difficult to understand. SCHERTZ (**29**) has shown that a draft on the nitrogen of the leaf by the younger tissues leads to a decomposition of nitrogen compounds of the leaf including proteins, phospho-lipines and chlorophyll, the decomposition of the latter producing mottling. With the loss of chlorophyll accompanying autumnal coloration, the yellow pigments increase at its expense due to the combination of the phytol that is split off from the chlorophyll with sugars to form carotin and xanthophyll (cited by SCHERTZ, *loc. cit.* p. 124). There can be no doubt, however, that whatever the cause, the present investigations confirm the existence of this migration phenomenon. The concurrent increase in nitrogen during this period in the short spurs, one year, two year and older branch growth,

which results from the evacuation from the leaves can readily be seen from an inspection of the analyses and fig. 1. The actual increase is probably greater than that shown if one allows for the relative increase of wood to bark. When the phenomenon of autumnal migration of nitrogen is considered in relation to the great demand of the shoots in the spring, as discussed above, this whole process may be looked upon as an expression of nature's method of conservation in the use of nitrogen.

THE PERIOD OF VEGETATIVE GROWTH.—Many investigators (**9**) have observed that a large decrease of nitrogen occurs in the older parts during the initiation of vegetative growth. Fig. 1 shows that during the period between the beginning of vegetative growth and the blossoming of the tree the loss of nitrogen from the branches is very large. The development of the buds and the formation of the new leaves are dependent upon the "reserve" proteins accumulated in the branches; a great expenditure of nitrogen compounds is thus required for growth of these parts. In some of the older tissues over half of the available stored nitrogen supply is given up to the young shoots, leaving a deficiency of nitrogen in these organs until this is supplied by the activity of the roots later in the spring.

A consideration of composite curves discussed elsewhere (**37**), showing the sum of the percentages of nitrogen in the leaves and in the one to seven year old branch growths, indicates that after *active* growth has ceased (third week in June) a condition of nitrogen equilibrium exists in the perennial parts until fall migration of nitrogen from the leaves commences.

CHANGES DURING THE DORMANT PERIOD.—The so-called dormant period is obviously accompanied by certain metabolic changes, for during this period with a mean daily temperature of 33.5° F. (the mean maximum being 35.5° F. and the mean minimum 20.2° F.) the total nitrogen in the short nonbearing spurs, one-year and two-year wood, decreased 11, 15 and 20 per cent., respectively.

During the commencement of the period of sap flow the nitrogen increased nearly 10 per cent. in the one-year wood and 18 per cent. in the two-year wood, and then during the rapid development of the shoot the nitrogen decreases rapidly in all parts due to the withdrawal of nitrogen stored in the phloem, as already stated. Compared with the quantities found when the tree entered the rest period, this amounts to a decrease of 32 per cent., 29 per cent., 21 per cent. and 22 per cent., respectively, in the short spurs, one-year branch growth, two-year branch growth, and older growth.

The form in which this decrease takes place cannot be measured by the changes in the water-soluble fractions taken at two periods only, because these are too small to account for the decrease involved. Apparently, there must be a continual degradation of the insoluble cytoplasmic proteins and

a transference to the older perennial parts throughout the whole dormant period.

### (b) Partition of nitrogen in the leaves

In the leaves, the fluctuations (see figures 2 and 3) of the water-soluble, the non-protein, and also of the free and combined amino-nitrogen fractions vary inversely to their course in the branch growth throughout the whole cycle. The amide N does not appear to follow this or any other definite relationship. This inverse relationship in the leaves and branches between the groups mentioned is difficult to interpret because, although it is possible to express the data from the leaves on both an absolute and a percentage basis, the results on the branch growth cannot be so stated unless the entire tree is taken for analysis and this is impracticable in experiments of this type. Fig. 3 shows that the total water-soluble, the free (mono- and di-amino), and also the free and combined amide nitrogen parallel the total nitrogen of the leaves throughout the cycle; the "rest" N usually varies inversely to them.

Although the amide N (free and combined) and also the "rest" N decrease in the fall during the period of migration of nitrogen from the leaves to the branches, the other fractions, *e.g.*, the total water-soluble, the

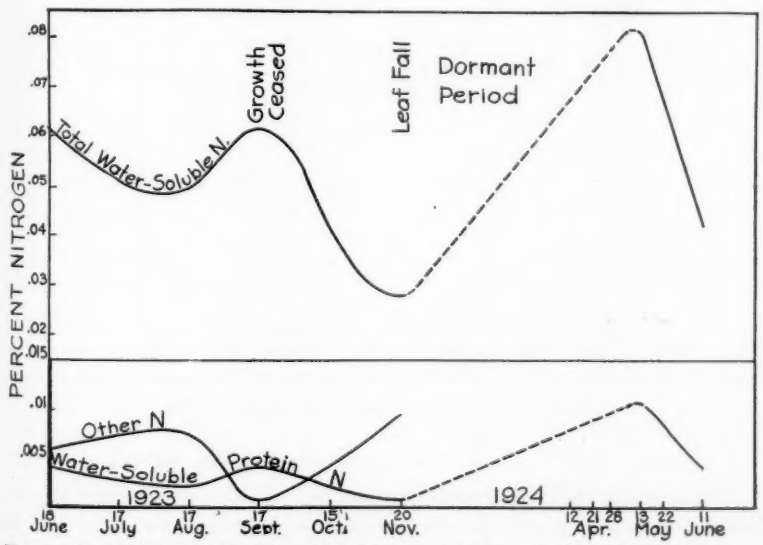


Fig. 2. Total water-soluble N, water-soluble protein N, and "rest" N of the leaves as percentages of the fresh weight.

non-protein, and the free (mono- and di-) amino-nitrogen decrease rapidly. The significance of this, to which reference will be made again, indicates that the "rest" N may have a close relation to protein degradation in the leaves (**3**). It is possible that nitrogen is translocated in the form of these "rest" N compounds. The water-soluble nitrogen is low throughout the cycle and varies inversely to the "rest" N. The significance of this is apparent.

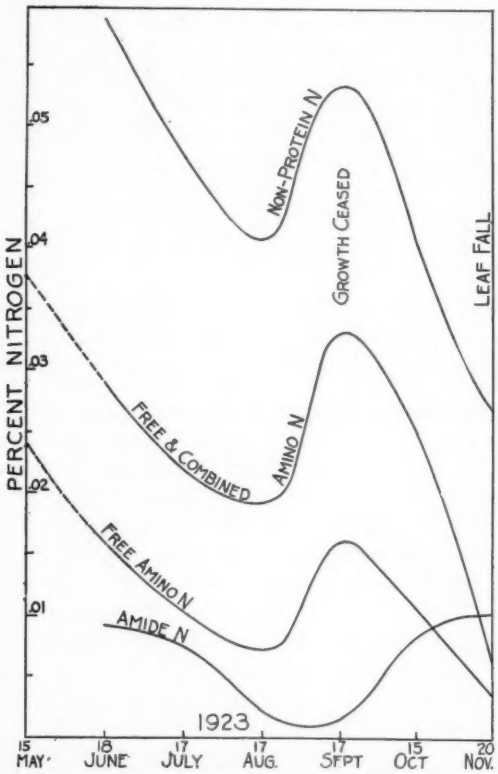


FIG. 3. The non-protein N, free and combined amino N, free amino N and amide N of the leaves as percentages of the fresh weight.

### (c) PARTITION OF NITROGEN IN THE BRANCH GROWTH

The decrease in total nitrogen in the one- and two-year branch growth during the period of mobilization of reserve proteins (bud swelling) is accompanied by a rapid rise in the total water-soluble non-protein nitrogen, in the free amino, and also in the "rest" nitrogen fractions, the amide N

decreasing during this period (fig. 4). Possibly the increase in the amino N at this time may indicate that translocation in the flowing sap takes place during this period as amino-acids. That the transportation of the reserve material to the tissues during the germination of wheat seedlings takes place in large measure as free amino-acids has been shown by PETTIBONE and KENNEDY (**25**) and is thus analogous to the manner in which the proteins of the blood stream are transported to the tissues of animals. However, the fact that the "rest" N also parallels the amino N in magnitude and direction at this period indicates the importance of the rôle that these unknown fractions may also play. Both CHIBNALL and PRIANISCHNIKOW (see ref. **43**, p. 660) affirm that asparagine is the form in which nitrogen is translocated from one part of the plant to another, but, as

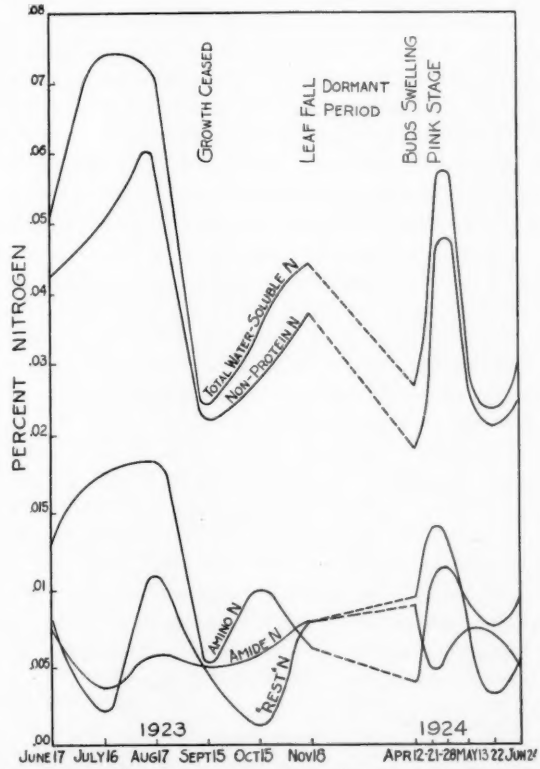


FIG. 4. Total water-soluble N, non-protein N, amino N, amide N, and "rest" N of one-year branch growth as percentages of the fresh weight.

pointed out by VICKERY (**43**), the presence of a number of other amino-acids found by him in appreciable amounts in alfalfa sap may indicate that the translocating processes are not so simple as that indicated by CHIBNALL and PRIANISCHNIKOW. It is expected that some additional light will be thrown on this subject later in the present series of investigation on *Pyrus malus*.

The sharp decrease in total nitrogen occurring in the one- and two-year branch growth and non-bearing spurs, between the beginning of vegetative growth and the period of blossoming, is accompanied by a rapid decrease in the total water-soluble, non-protein, and free amino nitrogen, but a gradual increase in the amide N. This seems to indicate that the material for the development of flowers was being drawn rapidly from the "reserve" proteins in the form of amides.

During the period of active growth, the water-soluble non-protein nitrogen, including the amino N, again increases. The fact that the amide N simultaneously decreases may indicate its connection with the utilization of amide N in protein synthesis. At times throughout the cycle the amide and amino N show inverse relationships. Since there is evidence (**34**) to indicate that in the germination of seeds amide N is formed from amino N, some such relationship may hold throughout in these experiments but may be masked at times by the difficulties involved in drawing sharp distinction between the groups. When active growth is over both the total water-soluble nitrogen and the non-protein nitrogen, including the amino and amide N, decline rapidly until the fall evacuation from the leaves commences. During this process of migration of nitrogen both the water-soluble and non-protein nitrogen increase up to the last sampling (November 20). The course of the fluctuation of the non-bearing spurs follows that of the one- and two-year branch growth.

During the so-called "dormant" period the amide N has increased but the amino N has decreased. A small amount (0.13 gm.) of a substance corresponding to the properties of asparagine was isolated from the 1923 dormant branch growth by a method to be given in a later paper.

Since asparagine contains both the $-CO \cdot NH_2$ and $-NH_2$ groups, the latter in the $\alpha$-position, it is clear that other amino-acids are (as would be expected) present; otherwise the amide and amino N would always parallel one another throughout the cycle. In a later investigation an attempt will be made to identify the amines and amides present.

## Summary

The quantitative changes of the principal nitrogen fractions in the non-bearing spurs and in the one- and two-year branch growths and leaves may be summarized:

I. Period of bud swelling

(*a*) ***Branches and non-bearing spurs.***—Total nitrogen, total water-soluble nitrogen, non-protein nitrogen, amino N and "rest" N increase; amide N decreases.

II. Period of commencement of vegetative growth

(*a*) ***Branches and non-bearing spurs.***—Total nitrogen, total water-soluble nitrogen, non-protein nitrogen and amino N decrease; amide N increases.

III. Period of active growth

(*a*) ***Branches and non-bearing spurs.***—Total nitrogen, total water-soluble nitrogen, and amino N increase; amide N decreases.

IV. Period of cessation of active growth

(*a*) ***Branches and spurs.***—Total nitrogen, total water-soluble nitrogen, non-protein nitrogen, amino N and "rest" N decrease; amide N increases.

(*b*) ***Leaves.***—For the first period to the middle of August, the total nitrogen, total water-soluble nitrogen, non-protein nitrogen, amino N and amide N decreases; the "rest" N remains unchanged. During the second period to the middle of September, all the aforementioned fractions increase except the "rest" N which decreases.

V. Period of chlorophyll degeneration

(*a*) ***Branches and non-bearing spurs.***—Total nitrogen, total water-soluble nitrogen, non-protein nitrogen and amide N increase; amino N decreases.

(*b*) ***Leaves.***—Total nitrogen, total water-soluble nitrogen, non-protein nitrogen and amino N decrease; amide N and "rest" N increase.

From these variations certain specific facts showing some of the more definite relationships have been discussed. These are:

1. The total nitrogen and the various partition fractions of the leaves vary with growth, decreasing when growth is very rapid. The total water-soluble nitrogen, the non-protein nitrogen and the amino N parallel the total nitrogen throughout the cycle; the amide N and the "rest" N tend to vary inversely as these fractions. Since the total nitrogen parallels the insoluble nitrogen and since the latter consists chiefly of the insoluble cytoplasmic protein (**33**), this may be regarded as supporting Chibnall's contention (**3**) that amino N is connected with protein synthesis and the "rest" N with protein degradation.

2. The total nitrogen and the partition groups of the "*wood*" *growth* (spurs and branches) also vary with growth, increasing when it is rapid.

The total nitrogen decreases rapidly from the period of bud swelling to the period of full bloom, and the amount of the decrease is indicative of the demands of the young shoots for nitrogen stored in the phloem.

3. The indications are that during bud swelling the reserve proteins are transported to the actively growing parts in the form of amino-acids.

4. There can be no question as to the phenomenon of autumnal migration of nitrogen from the leaves to the branches in this species. Storage is mainly in the one- and two-year growths.

5. In the aerial parts nitric N (nitrates) was found only at one period, *viz.*, in the buds just as they were opening.

6. In this species nitrates are transformed to amino-acids for the most part in the fine roots.

7. The soluble protein nitrogen is very small throughout the cycle.

8. Ammonia nitrogen is very low throughout the cycle.

9. Nitrogen equilibrium is just about maintained by the application of 5 lbs. of $NaNO_3$ to a 15-year-old Stayman Winesap tree growing in sod.

10. The importance of the possible rôle by the "rest" N compounds is pointed out. The results tend to confirm CHIBNALL's theory that they are connected with protein degradation.

11. Owing to the small quantities not only of soluble proteins but also of the total water-soluble nitrogenous products, this species is not suitable for investigations on the mechanism of protein synthesis.

The writer desires to thank Professors C. A. SHULL and H. W. POPP for suggestions and criticisms relating to the manuscript and to acknowledge the assistance of Mr. C. A. KERN in the determination of the imbibitional and hygroscopic water, and of MISS ETHEL GINGRICH in the computations of the analytical data.

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# THE CURRENT MINERAL NUTRIENT CONTENT OF THE PLANT SOLUTION AS AN INDEX OF METABOLIC LIMITING CONDITIONS*

B. E. GILBERT, F. T. MCLEAN AND W. L. ADAMS

(WITH SEVEN FIGURES)

## Introduction

The rate of metabolism at any time during the life of a plant is usually governed by one or more limiting conditions. These conditions limiting metabolism may be either chemical or physical in nature; either a limiting supply of raw materials for metabolism or some condition due to the physical state of the soil, or atmosphere which interferes with the assimilation and elaboration of these raw materials. In this paper attention is drawn to three cases of inhibited metabolism and the accompanying effects on the current mineral nutrient content of the plant solution during 1926. The conditions which were seemingly responsible for decreased metabolism were:

1. A decreased supply of available manganese in a neutral soil, resulting in a marked chlorosis in beets and spinach.
2. Limiting amounts of phosphoric acid and nitrogen as supplied to the soil in fertilizers.
3. Unfavorable weather or cultural conditions.

## Analytical methods

The methods discussed by GILBERT in an earlier publication (**2**) were followed with certain changes of procedure. It was found that when the plant solution was heated to 80° C. before filtering, flocculation took place and filtration was facilitated. Further work with the colorimetric method for potassium led to the adoption of the LINDO-GLADDING gravimetric method. This change was made due to the labor and time involved in recovering the waste platinum from the colorimetric method and also from the fact that by the gravimetric method it was found possible to determine accurately the amounts of potassium usually found in the plant solution.

## A limiting available manganese supply

For many years the Rhode Island experimental plats have grown crops which from time to time were distinctly chlorotic. It has been found that

* Contribution no. 345 of the Rhode Island Agricultural Experiment Station, Kingston, R. I.

this chlorosis occurs only with plants growing on soils which have been neutralized, and recently the correction of the conditions has been brought about by small applications of manganese salts in a spray (3), or as a fertilizer mixed with soil. Chemical determinations of the leafy tissue of crop plants have shown smaller amounts of total manganese in the chlorotic tissue than in the normal healthy green tissue (3). These results have led investigators to conceive the available manganese supply in the neutral or alkaline soil to be limiting and insufficient for normal metabolism. This condition proved to be most pronounced when coupled with a low nitrogen fertilization.

Plant solution determinations were made to compare the nutrient element levels in the chlorotic and non-chlorotic plants. In table I a comparison is given of the manganese fertilization and the nitrate-nitrogen as found in the plant solution.

TABLE I

COMPARISON OF NITRATE-N CONTENT OF PLANT SOLUTION AND MANGANESE FERTILIZATION

| CROP | TISSUE | PLAT | FERTILIZATION (LBS. PER ACRE) | | | | CONDITION OF PLANTS | NITRATE-N IN PLANT SOLUTION P. P. M. | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | N | $P_2O_5$ | $K_2O$ | Mn. | | | | |
| | | | | | | | | June 2 | | |
| Spinach | Leafy | 21 MG* | 0 | 200 | 30 | 11 | Green | 50 | | |
| Spinach | Leafy | 21 MG | 0 | 200 | 30 | 0 | Chlorotic | 283 | | |
| Spinach | Leafy | 50 MG | 60 | 200 | 30 | 9 | Green | 138 | | |
| Beets | Leaves and petioles | 119 MG | 45 | 120 | 90 | 9 | Green | Sept. 17 59 | Oct. 1 42 | Oct. 8 30 |
| Beets | Leaves and petioles | 119 MG | 45 | 120 | 90 | 0 | Chlorotic | 176 | 104 | 28 |
| Beets | Roots | 119 MG | 45 | 120 | 90 | 9 | Green | 200 | 283 | 125 |
| Beets | Roots | 119 MG | 45 | 120 | 90 | 0 | Chlorotic | 600 | 625 | 213 |
| Beets | Roots | 118 MG | 90 | 120 | 90 | 9 | Green | | 277 | |

* Market Garden Plat.

The application of manganese salts was accompanied by an increased yield as noted with previous crops (3) and also a decreased reserve of unmetabolized nitrogen as nitrate. The potassium reserve was likewise much reduced in the greener plants of spinach grown on plat 21. The manganese-treated plants contained potassium to the extent of 768 parts per million, while those from the untreated area showed 1,213 parts per million in the plant solution.

Considering the nitrogen situation with spinach on plat 21, it must be noted that in reality there were two limiting fertilizer conditions operating, *viz.*, insufficient manganese supply and a diminished nitrogen supply. This is seen when a comparison of yields of spinach is made between the manganese-treated areas on plat 21 and plat 50 (near plat 21 and had received nitrogen fertilization of 60 pounds per acre). Plat 21 was depressed 59 per cent. as compared to plat 50. This fact may help to account for the magnitude of the difference between the nitrate-nitrogen content of the plant solution on the treated and untreated areas of plat 21, the limiting nitrogen supply having accentuated the condition.

When beets growing on plat 119 are considered, however, nitrogen having been included in the fertilization, a comparison of yields between treated areas of plat 119 and plat 118 (adjacent and fertilized with 90 pounds of nitrogen per acre), shows the decrease of yield with decreased nitrogen to be only 26 per cent. Hence nitrogen as a limiting factor was less active. The nitrate-nitrogen difference between the treated and untreated beet plants is thus due more directly to the limiting manganese supply. The accumulation of nitrates both in the petioles and in the storage roots of beets with deficient manganese supply, is fully as marked as in the leaves of spinach. Only at the end of the growing season, on October 8, did the nitrogen content of the leaf petioles become equalized in the chlorotic, manganese-deficient plants and in the normal ones. This may be accounted for by the withdrawal of nitrogen from the old leaves, as there was still a relatively high nitrate content in the roots of the chlorotic plants on that date.

### Insufficient nutrients as metabolic limiting conditions

The most obvious conditions instrumental in limiting metabolism, are insufficient supplies of raw materials. Under surroundings such as occur in nature these are usually considered to arise from deficiency in or unavailability of the supplies of nitrogen, phosphoric acid, and potassium, as found in the soil in which the plant grows. When any one of these nutrients becomes limiting in supply, the limitation is reflected in the growth and may be measured by the yield at the end of the growth period. If, due to some such limiting nutrient supply a difference in growth or yield results, other conditions being the same, some reflection may be expected in the amounts of this nutrient in the plant solution. It may also be profitable to examine the relationship of another nutrient to the limiting one, as shown by the plant solution content.

In table II some data are given which show clearly the reflection in the plant solution of the amounts of nitrogen and phosphoric acid applied in fertilizer chemicals. An increase in either nutrient is shown in the current amounts of nitrate-nitrogen and phosphate-phosphorus.

## TABLE II

COMPARISON OF NITRATE-N AND PHOSPHATE-P IN PLANT SOLUTION WITH LIMITING SUPPLIES OF NUTRIENT APPLIED TO SOIL*

| CROP | TISSUE | PLAT | RELATIVE YIELD OF TISSUE | NITRATE-N IN PLANT SOLUTION P. P. M. | | | | | PHOSPHATE-P IN PLANT SOLUTION P. P. M. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | July 20 | Aug. 3 | Aug. 16 | Sept. 1 | Sept. 14 | July 20 | Aug. 3 | Aug. 16 | Sept. 1 | Sept. 14 |
| Turnip | Roots | 55N | 95 | 681 | 614 | 506 | 545 | 189 | 9.0 | 3.0 | 2.00 | 5.4 | 9.6 |
| Turnip | Roots | 65N | 100 | 535 | 545 | 580 | 268 | 395 | 50.0 | 34.0 | 13.8 | 20.8 | 11.7 |
| Turnip | Roots | 65S | 95 | 625 | 652 | 567 | 380 | 428 | 20.0 | 37.5 | 13.5 | 13.0 | 6.6 |
| | | | | July 16 | July 28 | Aug. 9 | | | July 16 | July 28 | Aug. 9 | | |
| Corn | One foot of stem above highest node | 55N | 92 | 340 | 319 | 113 | | | 33.3 | 26.8 | 1.00 | | |
| Corn | One foot of stem above highest node | 65N | 100 | 319 | 288 | 79 | | | 38.4 | 28.8 | 5.7 | | |
| Corn | One foot of stem above highest node | 65S | 100 | 405 | 394 | 102 | | | 40.0 | 37.5 | 6.9 | | |
| | | | | Aug. 20 | Aug. 31 | Sept. 13 | | | Aug. 20 | Aug. 31 | Sept. 13 | | |
| Cabbage | Lower leaves minus midribs | 55N | 54 | 250 | 155 | 97 | | | 0.86 | 4.45 | 1.38 | | |
| Cabbage | Lower leaves minus midribs | 65N | 94 | 256 | 169 | 40 | | | 1.11 | 5.20 | 1.56 | | |
| Cabbage | Lower leaves minus midribs | 65S | 100 | 325 | 187 | 88 | | | 1.18 | 1.15 | 0.75 | | |
| | | | | July 27 | Aug. 5 | Aug. 18 | Sept. 3 | Sept. 16 | July 27 | Aug. 5 | Aug. 18 | Sept. 3 | Sept. 16 |
| Carrots | Roots | 55N | 97 | 147 | 214 | 102 | 49 | 25 | 25.0 | 13.4 | 18.0 | 17.0 | 11.7 |
| Carrots | Roots | 65N | 100 | 100 | 137 | 69 | 71 | 76 | 31.0 | 15.5 | 19.7 | 21.5 | 14.7 |
| Carrots | Roots | 65S | 93 | 78 | 318 | 91 | 169 | 31 | 31.0 | 15.5 | 19.7 | 16.5 | 10.8 |

* Fertilizers applied as N: $P_2O_5$: $K_2O$: Plat 55 North 55: 50: 135 lbs. per acre.
65 North 55: 150: 135 lbs. per acre.
65 South 80: 150: 135 lbs. per acre.

The story as regards relative yields as measures of growth is, however, not so clear, and needs more explanation than is given in the table. With all the crops tested the increase in phosphoric acid as supplied between plats 55N and 65N is clearly shown both by the phosphate-phosphorus in the plant solution and by the relative yields. But when we compare relative yields of tissue of plats 65N and 65S, the latter of which received 25 pounds extra nitrogen per acre, we find that with turnips and carrots a decrease in yield was obtained in the case of 65S; with corn no difference resulted; while with cabbage there was an increase of 6 per cent. in the relative yields. If the particular tissue examined be considered, this variation in yield will be understood. With turnips and carrots the root tissue was analyzed while in the case of cabbage the leaves were used for analysis. The relative yields given in the table are of these tissues. If, however, the weights of turnip tops from plats 65N and 65S be expressed as relative yields they are 81 and 100 respectively. Thus the additional nitrogen fertilizer tended to produce leafy growth at the expense of the other portions of the plants. Nitrogen was limiting for leafy growth only, and as the tissue examined in the case of cabbage was leafy, this was reflected in the yield. With corn the stem tissue examined may be considered to have been intermediate and not likely to be greatly affected by the extra nitrogen of 65S.

Considering next the effect of the supply of one nutrient upon the plant solution content of another, we find that when the phosphoric acid applied and the phosphate-phosphorus are low, the nitrate-nitrogen in the plant solution is high and *vice versa*. This seems to hold with turnips, corn and carrots, but not with cabbage. In this latter crop, however, the phosphate-phosphorus differences between the plant solutions of plats 55N and 65N were small and may not have been great enough to affect the nitrate-nitrogen content. The reciprocal story is, however, not so clear. With the extra nitrogen applied and the higher nitrate-nitrogen content of the solution in the case of 65S, the phosphate-phosphorus was lower with turnips and cabbage only. The story is confused with the other two crops. It would be of interest to observe the effect of a larger increase in nitrogen upon the phosphate-phosphorus content of the plant solution.

### Changes in external conditions affecting metabolism

From the foregoing facts it is evident that differences in the nutrient level are reflected in the composition of the plant solution. There are also temporary changes in the concentrations of fertilizer elements in the plants. When such changes simultaneously affect the composition of the plants growing under a variety of conditions of soil fertility, then we may expect to find that they are due to some external condition. Therefore, a study was made

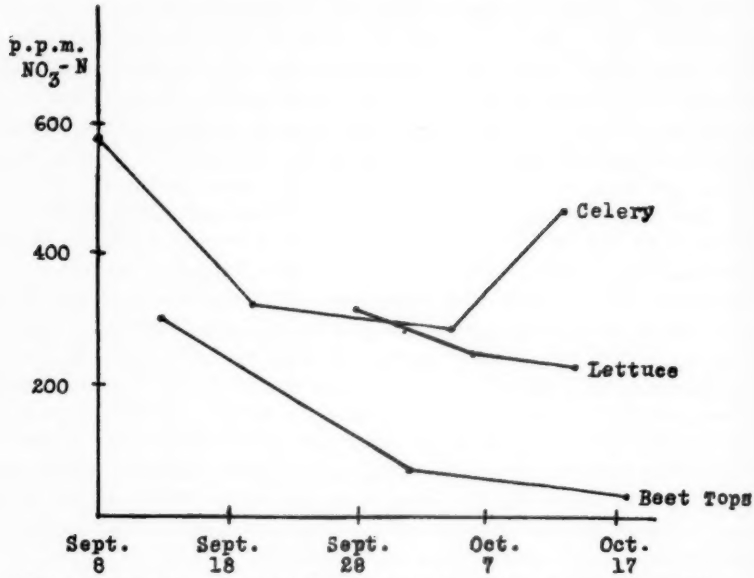


FIG. 1. Fluctuations in nitrate-nitrogen during decreasing temperature conditions.

of the weather conditions, and of the temperature and moisture content of the soil, to see if these factors could be correlated with changes in concentrations of the plant solution.

A conspicuous instance of this occurred about October 4 when the plant solution concentrations were very low. In figs. 1, 2, and 3 are shown graphical representations of the nitrate-nitrogen, phosphate-phosphorus, and potassium concentrations found at that time in celery, lettuce, and beet tops. The graph for each crop represents an average of determinations from two or more plats where nutrient levels were not limiting. It will be noted that with the exception of phosphorus in beet tops the three nutrient elements decrease in concentration through the month of September, and reach about a minimal value around October 4 for all nutrient elements and crops. A marked exception to this tendency for lowered concentration was noted in the case of late spinach which was planted on August 27 and showed a high nitrogen content on September 27.

The diminishing concentration of the plant solution of the crops was accompanied by a decreasing amount of nitrate-nitrogen in the soil.*

Except where large amounts of nitrogen were added during the month,

* Determinations by J. B. Smith, Assoc. Chemist.

## TABLE III

NITRATE-NITROGEN CONTENT OF THE TOP 7 INCHES OF SOIL UNDER GROWING CROPS (P. P. M.)

| Crop | Spinach | | Beets | | Celery | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Plat No. | 112 | 113 | 118 | 119 | 85 | 115 | 116 | 58 | 29 | 60 |
| Aug. 31 | ..... | ..... | 13 | 10 | 8 | 12 | 4 | 25 | 10 | 13 |
| Sept. 15 | 61 | 26 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Sept. 27 | 44 | 22 | 8 | 4 | 6 | 28[a] | 5 | 38[a] | 10 | 18[a] |
| Oct. 8 | 34 | 8 | 8 | 2 | 8 | 8 | 2 | 30 | 8 | 7 |

[a] Forty pounds of nitrogen per acre in nitrate of soda were added to each of these plats on September 24, three days before sampling. This quite probably increased the nitrate content of the soil in these plats on September 27.

the nitrate content of the soil was low, or at least decreased, from August 31 until October 8, under the crops of spinach, beets, and celery. Plat 112 of spinach and plat 58 of celery were the only ones which retained a high nitrate content through the month of September.

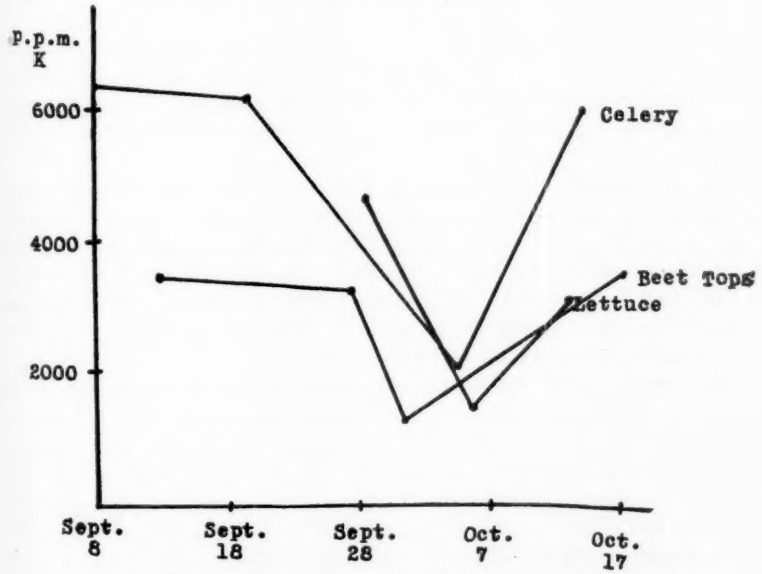


FIG. 2. Fluctuations in potassium during decreasing temperature conditions.

During the month of October the concentrations of the nutrient elements generally increased in the plant solutions of the three crops (figs. 1, 2, and 3).

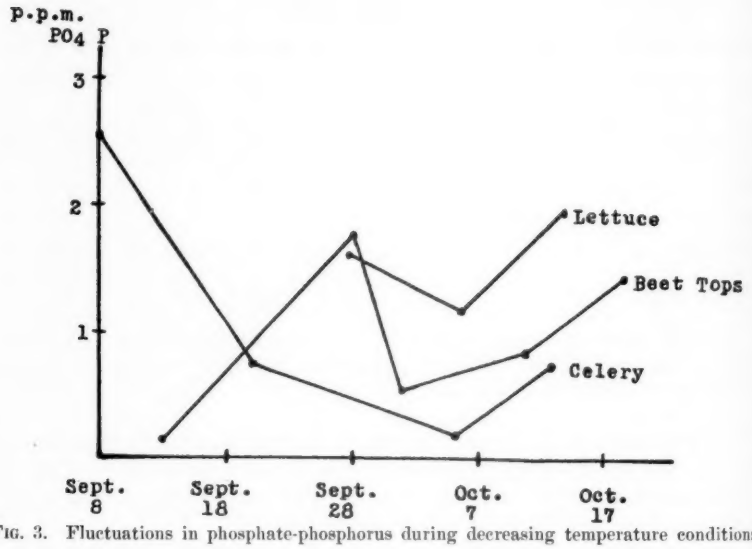


FIG. 3. Fluctuations in phosphate-phosphorus during decreasing temperature conditions.

These were accompanied by a marked decrease in both soil and air temperatures, as shown in fig. 7. This fall in temperature may have contributed to the accumulation of inorganic nitrogen, phosphorus, and potassium compounds in the plants, by depressing growth and decreasing the rate of metabolism.

The growth rates of the different crops studied were quite uniform throughout the period of most active growth, passing through a regular grand period of growth for each crop (figs. 5 and 6). Changes in weather and other changes of temporary character in the external conditions seemed to have little effect on the growth rate. The growth of every crop was markedly depressed by deficiencies of N, P, and K on plats which were purposely kept deficient in these fertilizer elements.

The only noticeable depression in growth increments which may be attributed to temporary unfavorable conditions for growth was during the period from July 15 to July 29. This showed as a depression in the growth of cabbage leaves as measured on July 22 (fig. 5). The index of leaf area used here in measuring the leaf growth of cabbage was the leaf product, obtained by multiplying the greatest width by the greatest length. This gave a value much larger than the actual leaf surface, but is believed to be approximately proportional to the leaf area (**4**), (**5**), (**6**).

There was a depression in height growth of turnips also, noted on July 29. Height of turnips was measured from the ground surface to the tip of the tallest leaf.

These two depressions in growth were correlated with notably high values for the nitrate content of the plant solutions; of cabbage on July 29, and of turnips and carrots on August 6 (fig. 4). This depressed growth of the plants and attendant accumulation of nitrate in the plant solution was accompanied by and followed a period of hot weather extending from July 15 to 25 (fig. 7).

The moisture content of the soil was not critically low at any time during the summer. It fluctuated between 20 per cent. and 35 per cent. of the dry weight of the soil. The moisture-holding capacity of these soils by the HILGARD test was from 36 per cent. for the mineral soils to 44 per cent. for the heavily-manured soils on the Market-Garden plats. Thus the calculated wilting coefficient of the soil by the BRIGGS and SHANTZ formula (1) was

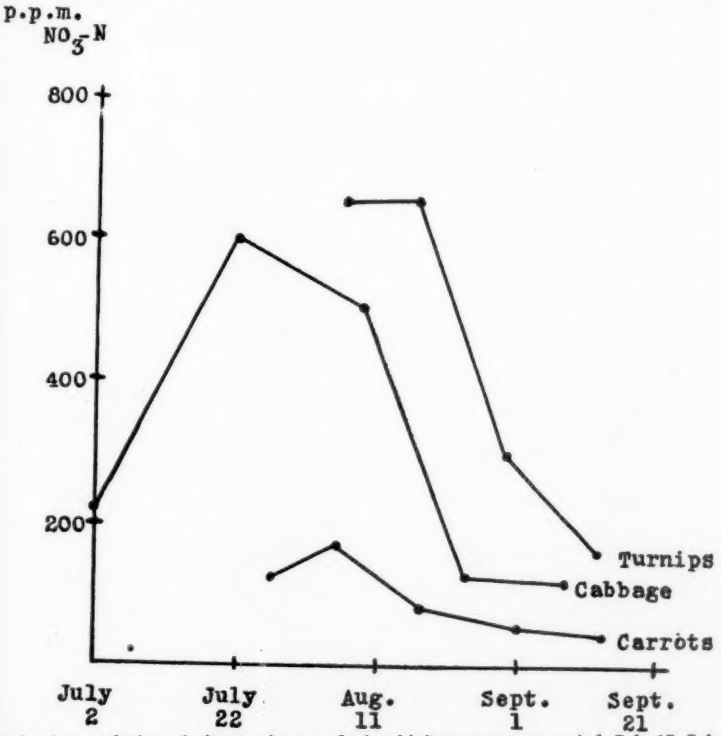


FIG. 4. Accumulation of nitrate-nitrogen during high temperature period, July 15–July 29.

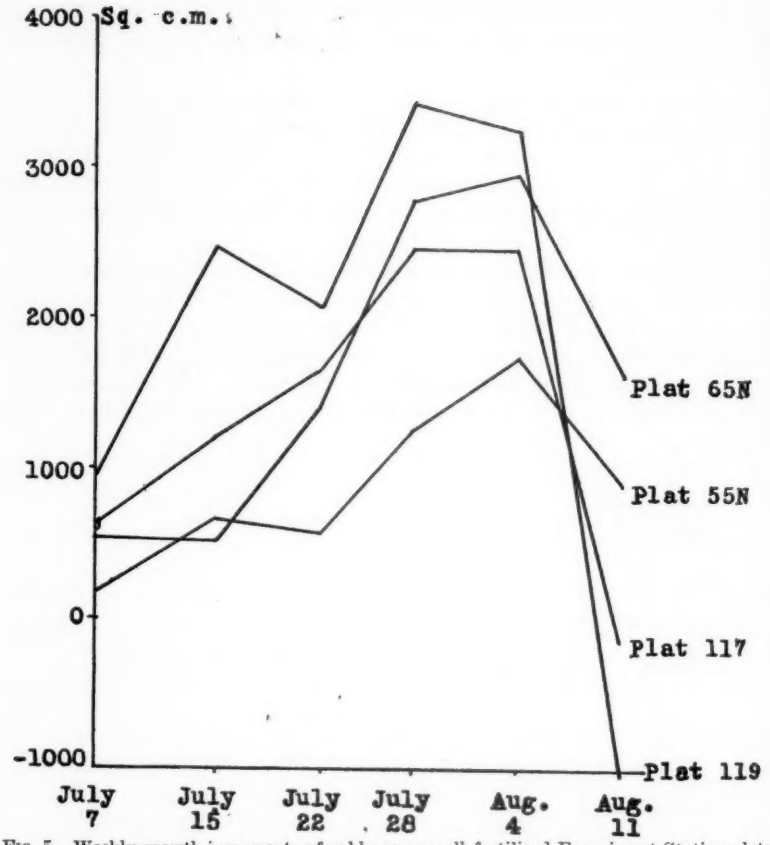


FIG. 5. Weekly growth increments of cabbage on well fertilized Experiment Station plats, 1926. Leaf product (length × width).

about 12 per cent., and the usually accepted optimum moisture content of the soil for plant growth—50 per cent. of the water-holding capacity—was approximately 18 per cent. to 22 per cent. So the moisture content of the soil does not appear to have been at all critically deficient at any time during the growing season.

## Discussion

The current mineral content of the plant solution has been shown to be an index of metabolic limiting conditions in three cases of inhibited

metabolism and these three cases have been discussed in turn. There now remains the developing of a concept to embrace limiting conditions in general. The authors have found it convenient to contrast the individual nutrient concentration in the plant solution with the level of water in the reservoir of a city supply system. This level is determined by the rate of inflow and the demands made upon the system by water consumption. In the plant, the concentration of any one nutrient element is determined by the available supply of that element in the soil and the permeability of plant membranes to the nutrient element. These govern the rates of inflow. At the other end of the system, use of the individual nutrient element is determined by the rates of metabolic processes, which are subject to changes through the action of limiting conditions. A further factor which may be expected to affect the concentrations is the amount of moisture present in the plant tissues. As this decreases with increasing saturation deficit, the plant solution may be expected to become proportionately more concen-

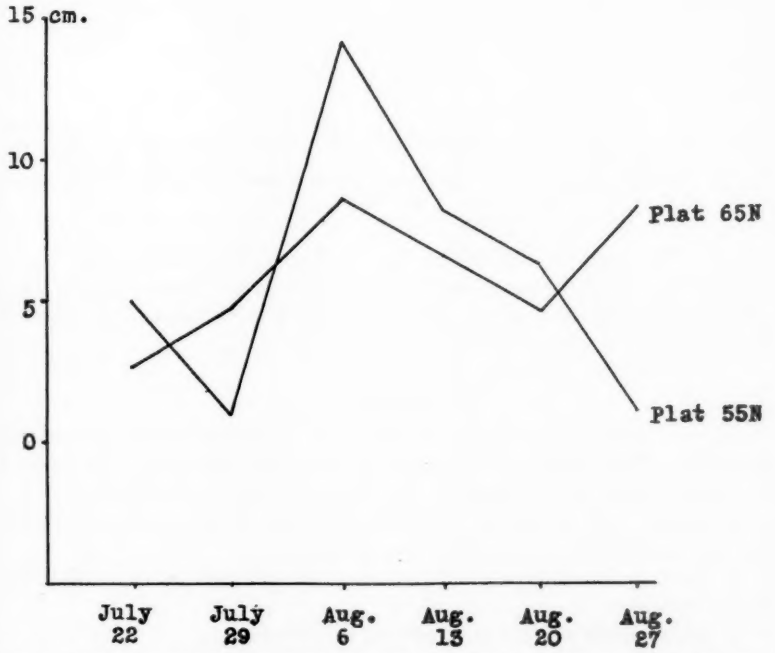


FIG. 6. Weekly growth increments of turnips on well fertilized Experiment Station plats, 1926. Height of tallest leaf in cm.

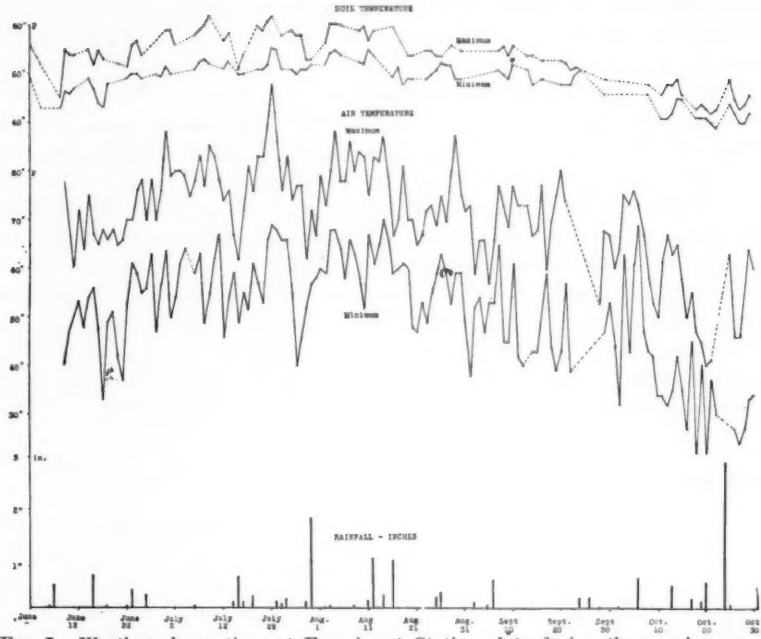


FIG. 7. Weather observations at Experiment Station plats during the growing season of 1926.

trated. The extent of this effect has not been determined as yet, but it may be expected to be small except under severe conditions of evaporation, when it also would assume the rôle of a limiting condition and be reflected in the current nutrient-element concentration.

## Summary

In this paper the current amounts of nitrate-nitrogen, phosphate-phosphorus, and potassium are shown to have been correlated with arrested metabolism which was induced by the following limiting conditions:

1. A decreased supply of available manganese in a neutral soil, resulting in a marked chlorosis in beets and spinach.

2. Limiting amounts of phosphoric acid and nitrogen as supplied to the soil in fertilizers.

3. Unfavorable weather and cultural conditions.

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# GROWTH STUDIES ON FRUITS
## AN EXPLANATION OF THE SHAPE OF THE GROWTH CURVE*

F. G. GUSTAFSON

(WITH THREE FIGURES)

In a recent paper the writer (**6**) has shown that the growth curves of fruits are similar to those of animals and vegetative parts of plants. This has also been shown by ANDERSON (**1**) and GOLINSKA (**4**). Some of the data used in the previous paper were obtained from fruits set at different times and it was suggested that it might be more accurate to have a large number of plants in blossom at the same time and on these tag several thousand blossoms growing in identical positions on the plants. Collections for volume and weight determinations could be made from these every week or more often if desired.

This was done during the summer of 1926. Figs. 1 and 2 show the result of this experiment. The volume and weight measurements were made every other day for the first three weeks and after that every week. The number of fruits used varied from 200 at the time of setting to 50 from the ninth day to the end. A conscious attempt was made to pick average fruits each time. These fruits were all weighed together and the average individual weight obtained. Their average individual volume was obtained by finding the volume of water displaced by them. The volume and green weight curves are almost identical.

As everyone knows who has studied growth, ROBERTSON (**13**) and OSTWALD (**9**) independently were the first to liken growth to an autocatalytic reaction of the first order, which also gives an S-shaped curve. Both of these writers, using their own data as well as that of other investigators, compared the observed growth curves with the calculated curves, using the monomolecular autocatalytic equation. Both report close agreements, as have other investigators since.

Recently, however, GREGORY (**5**) maintains that only the latter half of the growth curve of barley can be represented by an autocatalytic reaction equation. VAN DE SANDE-BAKHUYZEN (**2**) states that the calculated and observed values do not agree at all. Thus we see that on the basis of calculation, two investigators have during the last year denied that the ROBERTSON and OSTWALD formula holds for the growth of plants.

* Paper from the Department of Botany of the University of Michigan, no. 256.

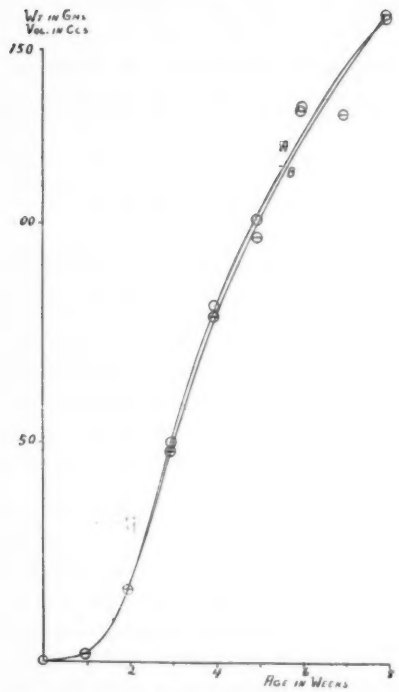


FIG. 1. Growth curves of tomato fruits. Volume and green weight determined weekly, from 200–50 picked fruits, previously tagged at the same time. Curve A, average volume of one fruit; curve B, average green weight of one fruit.

There are many investigators who explain growth in other ways than by autocatalysis. This is particularly true of investigators working with unicellular organisms such as bacteria and yeast. Thus MCKENDRICK (7), studying bacteria, came to the conclusion that "The rate of multiplication of fast-growing micro-organisms is proportional to the number of organisms and to the concentration of food stuffs."

SLATOR (15) has found that "If the seeding is small and all necessary food for yeast growth is in excess, the growth during the earlier stages of the reaction is unrestricted and follows the logarithmic law of increase, that is the rate of increase is always proportional to the quantity present. At a later period retarding influences come into action, the yeast multiplication becomes restricted and during the final stages of fermentation ceases entirely."

PRIESTLEY and PEARSALL (12) explain the growth of roots of *Tradescantia zebrina* and *Lycopersicum esculentum* on the basis of food supply. They

noted several S-curves in the total growth of the root system and the flattening out of the curve seemed to be associated with the formation of the secondary or tertiary roots. PEARSALL (**10**) in a later paper has elaborated the theory that food supply is a factor in the growth curves of roots.

MURNEEK (**8**) explains the vegetative growth in tomato on the basis of correlation. He believes that the presence of fruits on a vine slows down the growth and finally inhibits the vegetative growth entirely. He shows that by the removal of the fruits the growth does not slow down but continues at the same rate as before.

From my own studies on the fruits of cucumber, summer squash, muskmelon and especially tomato I am inclined to believe that growth of fruits is mainly a matter of nutrition. By nutrition is meant the supply of food, mineral material, water, oxygen and any other material used in building up and maintaining the cells. This is the same idea that PRIESTLEY and PEARSALL have made use of in explaining root growth, applied to the growth of fruits.

If for a moment we go back to SACHS's experiments (**14**) on growth of roots of *Vicia faba* and the stem of *Phaseolus multiflorus* and plot his data as usual, *i.e.*, total length against time, we will obtain the typical S-shaped curve. In the experiment a disk of the stem or root 1 mm. thick undergoes

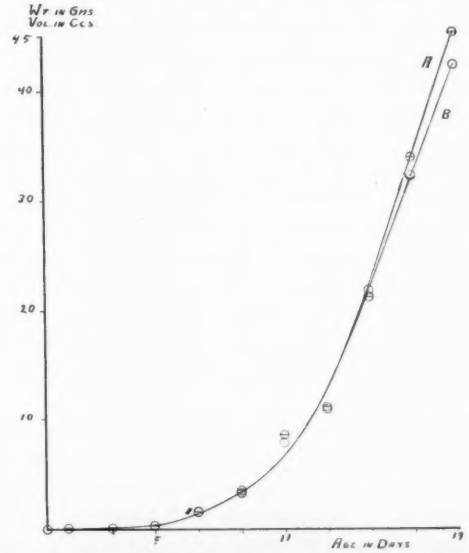


FIG. 2. First 19 days of growth of fruits shown in fig. 1. Measurements made on alternate days. First reading from 200 ovaries; later readings from decreasing numbers, 50 on the ninth day and subsequently. Curve A, volume; curve B, green weight.

a growth cycle which corresponds to that of a whole organism, or an organ of such an organism. This growth is made up of the cell formation (cell division), cell enlargement, and sometimes a further increase in mass brought about by the thickening of the cell walls or deposition of stored food material. In cell division there is very little if any increase in size, because the two cells will occupy the space of the one. However, when the meristematic cell enlarges preparatory to the division there probably is a slight increase. After the new cell has been formed, it begins actively to increase in size due to intake of water. This is at first not very rapid but it becomes more and more so. However, a time comes when not so much water is absorbed due to the fact that the forces which tend to keep the cell from further enlargement are nearly equal to the forces causing the water to enter the cell. When the two are equal there will be no further increase in size of the individual cell and growth as illustrated by increase in volume will cease. After the cell has stopped increasing in size there may of course be a thickening of the cell wall and even a storing of food material so that the mass of the cell would continue to increase for some time after the volume increase ceased. In fruits the increase in volume and dry weight are very closely associated as shown in the previous paper and in fig. 1 of this paper.

The growth of an organism is the sum of the growth of its individual cells. If in unit time, the same number of cells were formed and they increased to the same volume and mass during the whole life of an individual the rate of growth would be constant, but that is not a fact. In the young individual there are few cells either being formed or increasing in volume or mass. Consequently the actual increase in size must be very slow. If each cell kept on dividing at the same rate we would have a geometric rate of growth, which is approximated by the early part of the growth period. This period is followed by another in which the growth is proportional to time, and in turn this period gradually changes into one where there is very little and finally no growth at all. This may be explained by assuming that the number of cells formed gradually becomes smaller and finally ceases altogether or else the final size reached by each cell is less in the later life of an organism than in its early life. There is no evidence for the latter supposition in tomato fruits.

The question may fairly be asked, why does cell formation stop as the organ or organism becomes old and mature? My answer would be that there is a lack of nutrient material, *i.e.*, foods, minerals, water, etc.

For an elaboration of my thesis I wish to use as an illustration the tomato fruit. There is no question but that an available supply of nutrient material influences the size of the fruits to a great extent. It is a well established practice to thin the fruits, when the setting has been very heavy, to

obtain fewer but larger fruits. In the case of tomato it was noted in the paper cited that the first fruits to set on a plant are smaller than those setting later, when the plant is larger and more vigorous. If the plant is prevented from setting fruit until it is large, the first fruits will be large. The second fruit in a fruiting cluster is usually smaller than the first and the third is smaller than the second. Occasionally the second fruit is larger than the first, but this happens only when for some reason or other the first fruit does not begin to grow until the second fruit is of some size. The explanation presumably is that the second fruit got such a start that the main food supply is directed in that direction and it is so to speak on the main line of supply, while the first fruit has been shunted to the side line. If one examines such a cluster, it is at once noticed that the peduncle has straightened out in such a way that the second fruit is actually in a more direct line to the stem than the first fruit, which is now on a side branch.

It has been noted that when the first fruit is removed, the second, if it has not already commenced to ripen, will begin to grow very rapidly and will usually attain the same size as number one. I do not believe this is due to any inhibiting substances, of which we have heard so much, but of which we know so very little, because in the case of fruits setting on a small and young plant the first are always smaller than those setting later when the plant has more food material. If there were inhibitors, the later fruits ought to be smaller than the first, because they would be inhibited by the fruits first formed, while there would be nothing to inhibit the first fruits set except lack of material from which to grow.

If for a moment we assume that food supply is the factor influencing the rate of growth, how can we explain the shape of the growth curve? When the fruit is set there is an abundance of nutrient material for its needs (not much being needed), but it increases in size very slowly, because there are few cells dividing and enlarging. Later, as more cells are produced and begin to enlarge, the increase in size becomes more pronounced. For this rapid growth more nutrient material is needed and a time will come when there is not enough and the growth cannot go on increasing as it has, and we have a decline in rate. The reason the nutrient material becomes the limiting factor is partly due to the fact that there is not enough in the plant for all the fruits as well as for the vegetative growth and perhaps partly due to poor conduction of material to the growing part of the fruits. Consequently there is a decrease in the rate of growth and when total volume is plotted against age we get an S-shaped curve.

It is a well known fact that, if a few fruits are permitted to mature, the individuals will be larger; yet they will not grow indefinitely, but will eventually reach a limit beyond which they cannot be made to grow, even if only one fruit is produced by the plant. An explanation for the limit

of the size to which a fruit can grow, I believe, is connected with the conduction of materials into the fruit. The conductive system of a fruit like the tomato does not enlarge in proportion to the pulpy part. In the young fruit or in the ovary the vascular strands are fairly numerous and well developed, but the mature or nearly mature fruit is poorly supplied with such structures. For this reason, together with the fact that as the fruit becomes larger the amount of nutrient material needed also increases, it seems reasonable to assume that the conducting system should become inadequate. In the large fruits the food, water, oxygen and mineral material all have to be conducted by diffusion for much longer distances than in the young fruits. Even though the nutrient gradient may be quite high the difficulty of transportation is so great that eventually the fruit stops increasing in size entirely. It is to be remembered that even though the fruit does not increase in volume or mass, yet material must be taken into it unless it is actually to decrease in size. This is true of water at least. Muskmelons and summer squashes growing in bright sunlight and in a dry field may actually decrease in volume considerably while maturing.

The growth of the shoot of a plant can also be explained on the basis of nutrition. When a seed germinates it is supplied with an abundance of nutrient material, but the cells growing are few, and the increase in mass of the plant is very slow at first. In fact, as BRENCHLEY (**3**) has shown, and as found by the author in experiments not yet published, there is in some plants an actual decrease in dry weight for the first two or three weeks, even though the volume increases very extensively. As time progresses, the number of cells growing increases, and the food supply is augmented by the photosynthesis of the leaves. However, not all cells formed become part of the manufacturing tissue nor of the absorptive tissue. For every cell that goes to form chlorenchyma or root tissue there are others that go to form stem, veins in the leaf, and later to form the flowers and the fruits. Yet all of these cells depend upon the cells of the chlorenchyma and of root tissues for their nutrient material. Thus the proportion of nutrient consuming tissue to the nutrient obtaining tissue increases as the plant ages.

MURNEEK (**8**) has shown that when fruits of the tomato are prevented from forming, the vegetative part of the tomato plant keeps on growing at a more or less constant rate. POPP (**11**) has also noted that when Biloxi soy beans grown under artificial light conditions are prevented from blossoming the plants keep on growing and the curves obtained are not of the sigmoid type.

The writer conducted an experiment with a variety of pole bean in which a part of the plants were deflorated while other plants were permitted to blossom and fruit. The seeds were planted May 24, and the first measurements were made two weeks later. From that time, measurements were

made weekly except for the tenth week. The weather was very dry during the first eight weeks and the last three of those weeks were also very hot. This probably accounts for the very slow growth during that period. On July 27, nine weeks after planting, the plants were divided into two groups. From that time one group had all the blossoms or flower buds removed as soon as they appeared in an endeavor to prevent any fruits from forming; the other group formed fruits in the usual way. Fig. 3 shows that the

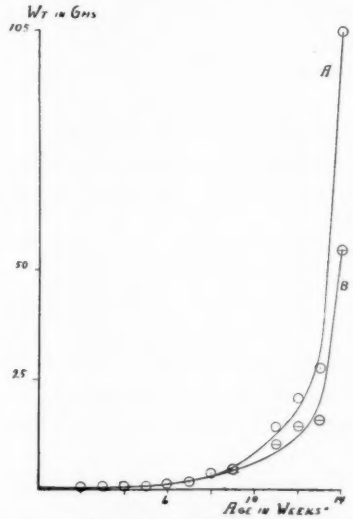


FIG. 3. Curve of increased dry weight for pole bean, in two groups after the ninth week. Curve A, deflorated plants; curve B, fruiting plants. Average final dry weight of plants on curve A, 105.21 gm.; curve B, 55.48 gm.

plants which did not fruit formed a much greater amount of dry material than those which fruited. Not enough plants were available to carry on the experiment till the plants with fruits stopped growing; but as the point of the experiment was to show that when fruits did not have to be supported by the plant the vegetative portion would grow much longer, it was conducted far enough to demonstrate this point clearly.

These illustrations show that when the nutrient material was not diverted from the shoot the total size and dry weight increased much more than when part of the nutrient material was diverted to fruits and flowers. One explanation for this would be that more nutrient material was available for the formation of new leaves, which in turn would increase the dry weight of the plant by photosynthesis.

## Summary

1. This paper discusses in some detail the probable influence of nutrition upon the form of the growth curve of fruits. It is also pointed out that the poorly developed conductive system in fruits may very materially limit the nutrient material supplied to the fruits.

2. It is further pointed out that when nutrient materials are not diverted to fruits, the rate of growth of the shoot continues at an approximately uniform rate, rather than at an increasingly lower rate as is usual when fruiting takes place.


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# CHEMICAL CHANGES ACCOMPANYING THE WESTERN YELLOW BLIGHT OF TOMATO*

J. T. ROSA[1]

The disease of the tomato plant called Western Yellow Blight is frequently very destructive in western North America but is not known to occur elsewhere. The true nature and the cause of the disease have baffled pathologists for 30 years. It was thought that an examination of the changes in plant composition connected with the disease might throw some light on its nature.

The symptoms of this blight have been adequately described by SHAPOVALOV (7). Briefly, vegetative growth ceases; the leaflets become leathery and the margins roll upward; the stems, petioles and leaf veins become purpled, and the upper surface of the leaf becomes more or less yellow. Affected plants are non-productive and, usually die after a few weeks. Occasionally, however, regeneration of part or all of a blighted plant occurs in the field. Efforts by the writer to induce regeneration by cutting back the tops, and by spraying with nitrates and compounds of iron have given negative results. Stem cuttings of blighted plants placed in tap water and in nutrient solutions often produced roots but no vegetative growth.

For analytical purposes, samples were collected at Davis in June, 1925, of roots, stems and leaves from healthy and blighted plants of the Earliana variety. Other samples were collected in June and July, 1926, of the San Jose Canner variety. All samples were taken about 11 A. M., during clear hot weather. The samples of June 8, 1925, were taken at the beginning of an attack that killed 95 per cent. of the plants within the next few weeks; those of June 18, 1925, were of plants that had been definitely blighted for ten days or more. The samples of 1926 were taken at the beginning of two mild attacks which together destroyed only 5 per cent. of the plants. The sample designated as "incipient blight" showed slight external indications of the disease for several weeks. It eventually recovered, however, and bore a normal crop.

The samples of January, 1926, are of special interest. These were taken at Riverside in Mr. SHAPOVALOV'S plots. The plants were those of the preceding summer's crop, which had escaped a moderate attack of blight,

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[1] Acknowledgment is made to F. S. HENIKA for performing the analytical work.

and due to absence of frost continued growth till midwinter. All of the plants in this field, except in 2 plots, assumed in early winter the yellowish-purple color and the non-vegetative condition characteristic of blight. One of the exceptional plots was fertilized with sulphate of ammonia, the other received seepage from a leaky sewer. The plants in these plots were dark green and vigorously vegetative. Because of the known deficiency of nitrogen in the soil, these latter have been termed "ample nitrogen" plants, the former "nitrogen starved" plants. Since the nitrogen starved plants had the external appearance of blight, it was of interest to determine if this condition could be differentiated chemically from true blight.

Plant samples were preserved in boiling 95 per cent. alcohol (with CaO). After drying the solid portion of the sample, and grinding, extraction was completed with 50 per cent. alcohol. Carbohydrates were determined by the SCHAFFER-HARTMAN method—sucrose after inversion with invertase (1 cc. in 100 for 2 hours), and starch after autoclaving for one hour and digestion with taka-diastase over night. Total nitrogen was determined by the official salicylic-sulphuric acid method, observing the precautions advised by RANKER. Nitrogen was determined separately on aliquots of the insoluble and alcohol-soluble portions of each sample. The per cent. of the total nitrogen occurring in the soluble fraction is also given. The amino nitrogen in the alcohol extract was also determined but the results are not presented; in general amino nitrogen constituted about one fourth of the alcohol-soluble fraction.

Table I gives the changes in absolute amounts of certain constituents in healthy and blighted plants. It is evident that the Western Yellow Blight of tomato is characterized by very definite changes in composition. One of these is the increased percentage of dry matter in all parts of blighted plants. The changes in relative amounts of the different constituents (as shown when the results are calculated to the dry weight basis), are somewhat smaller than those given on the fresh weight basis. However, it is readily seen that there are marked changes in composition, both relative and absolute.

In the leaves, reducing sugars, sucrose, and starch increase progressively with the development of external symptoms of blight. It is probable that some of the striking symptoms of blight, *e.g.*, purpling of stems and veins, and rolling of the leaves, are connected with the high carbohydrate content. The purple pigment, anthocyanin, is well known to become intensified in the presence of high sugar concentration.

There is a very decided decrease of total nitrogen in blighted leaves; this decrease is entirely in the insoluble fraction. In fact, the amount of soluble nitrogen actually increases, and the per cent. in soluble form in-

TABLE I

COMPOSITION OF HEALTHY AND BLIGHTED TOMATO PLANTS—AS PERCENTAGE OF FRESH WEIGHT

| DATE | MATERIAL | DRY MATTER | REDUCING SUGAR | SUCROSE | STARCH | NITROGEN | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | TOTAL | INSOLUBLE | SOLUBLE | PER CENT. SOLUBLE |
| Leaves | | | | | | | | | |
| June 8, '25 | Healthy | 15.1 | 0.62 | 0.39 | 1.11 | 0.608 | 0.518 | 0.091 | 14.9 |
| June 8, '25 | Severe blight | 18.6 | 1.91 | 0.73 | 3.52 | 0.440 | 0.320 | 0.120 | 27.3 |
| June 18, '25 | Very severe blight | 18.9 | 3.19 | 1.36 | 2.18 | 0.457 | 0.302 | 0.155 | 33.9 |
| June 18, '26 | Healthy | 15.0 | 0.38 | 0.20 | 2.25 | 0.675 | 0.576 | 0.099 | 14.6 |
| June 18, '26 | Incipient blight | 16.1 | 0.65 | 0.09 | 3.61 | 0.595 | 0.481 | 0.114 | 20.0 |
| June 18, '26 | Severe blight | 20.1 | 1.40 | 0.38 | 6.48 | 0.485 | 0.341 | 0.144 | 30.5 |
| July 24, '26 | Healthy | 12.5 | 0.78 | 0.10 | 1.23 | 0.585 | 0.495 | 0.090 | 15.4 |
| July 24, '26 | Recently blighted | 15.9 | 1.21 | 0.25 | 3.42 | 0.414 | 0.303 | 0.111 | 26.8 |
| Jan. 8, '26 | Ample nitrogen | 12.7 | 0.18 | 0.29 | 2.85 | 0.557 | 0.441 | 0.116 | 20.9 |
| Jan. 8, '26 | Nitrogen starved | 18.5 | 1.12 | 1.08 | 7.42 | 0.482 | 0.400 | 0.082 | 17.0 |
| Stems | | | | | | | | | |
| June 8, '25 | Healthy | 9.6 | 0.94 | 0.31 | 0.74 | 0.201 | 0.119 | 0.083 | 41.3 |
| June 8, '25 | Severe blight | 13.9 | 2.06 | 1.15 | 3.41 | 0.279 | 0.161 | 0.117 | 42.0 |
| June 18, '25 | Very severe blight | 15.0 | 2.31 | 1.36 | 1.22 | 0.372 | 0.196 | 0.177 | 47.6 |
| June 18, '26 | Healthy | 8.0 | 0.35 | 0.06 | 0.37 | 0.231 | 0.085 | 0.146 | 63.2 |
| June 18, '26 | Recently blighted | 11.0 | 1.85 | 0.92 | 1.10 | 0.284 | 0.127 | 0.154 | 54.2 |
| Roots | | | | | | | | | |
| June 8, '25 | Healthy | 15.3 | 0.35 | 0.74 | 0.82 | 0.280 | 0.183 | 0.096 | 34.4 |
| June 8, '25 | Severe blight | 17.1 | 0.65 | 1.42 | 1.18 | 0.389 | 0.256 | 0.133 | 34.2 |
| June 18, '25 | Very severe blight | 15.6 | 0.70 | 2.44 | 2.34 | 0.387 | 0.176 | 0.211 | 54.4 |

creases markedly. It seems as though upon the inception of blight, the movement of nitrogen into the leaves is stopped before growth is entirely halted, thus diluting the nitrogen already present and reducing the per cent. of nitrogen in these tissues. The accumulation of carbohydrates and other solids is not sufficient to account for the reduction in per cent. of total nitrogen. There seem to be other changes, also, which result in some of the more complex insoluble nitrogen constituents being converted back to soluble forms.

In the stems and roots of blighted plants, the accumulation of sugars and starch is almost as marked as in the leaves. Hence it is apparent that the disease involves all parts of the plant. It also seems that translocation of carbohydrates is not interfered with, but that their utilization in tissue-building is. The accumulation of carbohydrate is due mostly to the stopping of growth by some other unknown agency. While carbohydrate accumulation gives to the blight disease some of its characteristic symptoms, it is probably not the primary cause of the trouble.

In contrast to the leaves, total nitrogen in stems and roots shows an increase in the case of blight, this increase being especially noticeable in the roots. However, the proportion of soluble nitrogen increases in these tissues as it does in leaves, but not at the expense of the insoluble fraction. It appears, then, that blight is not due to nitrogen deficiency, for blighted plants continue, at least in the early stages of the disease, to take up nitrogen from the soil solution, though they are not able to translocate it into the leaves.

Attention may now be called to the two leaf samples taken in January at Riverside. The nitrogen starved plants, which externally had the appearance of Western Yellow Blight, likewise showed the extreme accumulation of sugars and starch characteristic of blight. As would be expected, total nitrogen is also somewhat decreased in the nitrogen-starved plants. However, the soluble nitrogen is also decreased somewhat, as is also the proportion of total nitrogen in soluble form, compared to that in the "ample nitrogen" or normal plants. Also the amino nitrogen is decreased from 3 per cent. of the total N. in normal to 1.9 per cent. in starved plants. Hence it seems that blighted tomato plants may be distinguished from those whose growth is checked by other causes, through their nitrogen relations.

It was thought that the starch accumulation in blighted plants might be connected with the inactivation of the natural plant diastases, hence determinations were made of the diastatic activity of healthy and blighted plants. Fifty grams of fresh ground tissue were extracted with 75 cc. glycerine (+ toluol) at 12° C. for 24 hours. This was made to 250 cc. and filtered. Aliquots of the filtrate were used to determine diastatic activity.

To one aliquot, 25 cc. of 1 per cent. soluble starch was added. Another aliquot, without starch, was run as a check, under similar conditions. After incubating 48 hours (24 hours in second experiment) at 30° C., the solutions were cleared and sugars determined. Gain in reducing sugar indicates diastatic activity in the plant material. The amounts are expressed as milligrams of dextrose, in table II.

TABLE II

DIASTATIC ACTIVITY OF LEAVES OF HEALTHY AND BLIGHTED PLANTS

| FIRST EXPERIMENT, JUNE 18, 1926 | EXTRACT + STARCH | EXTRACT ALONE | GAIN |
|---|---|---|---|
| | mg. | mg. | mg. |
| Leaves of healthy plants | 491.0 | 153.5 | 337.5 |
| Leaves of incipient blight | 591.0 | 396.0 | 195.0 |
| Leaves of severe blight | 1000.0 | 898.0 | 102.0 |
| SECOND EXPERIMENT, JULY 24, 1926 | | | |
| Leaves of healthy plants | 223.7 | 145.6 | 78.1 |
| Leaves of blighted plants | 506.8 | 483.7 | 23.1 |
| Stems of healthy plants | 278.7 | 270.6 | 8.1 |
| Stems of blighted plants | 603.1 | 603.1 | 0. |

It appears that diastatic activity is greatly reduced in the leaves of blighted plants, but does not disappear entirely. In healthy stems diastatic activity is surprisingly low, and it seems to have disappeared in blighted stems.

## Discussion

As previously stated, the cause of Western Yellow Blight is not known. SHAPOVALOV (**7**) has shown that the regional as well as the seasonal occurrence of the disease is associated to a very marked degree with weather conditions. Severe losses from blight occur under conditions conducive to high evaporating power of the air. SHAPOVALOV also records the favorable effect of shading upon the inhibition of blight, under conditions where otherwise it would have been very destructive. The writer has also observed cases where slight shading, as from young orchard trees, reduced the amount of blight. Since the evaporation rate would be little influenced by such transitory shading, some connection with the light factor is indicated. The disease generally appears in summer when the days are long, and in regions where the sunlight is particularly intense because of the cloudless days and clear atmosphere. The work of ARTHUR *et al* (**1**) and of Miss PFEIFFER (**6**) is also of interest in this connection. These workers found that tomatoes

grown with varying length of day evidenced injury with a daily light period of 17 hours or longer, indicating that the tomato is particularly sensitive to light. Miss PFEIFFER's long-day plants were dwarfed, yellowish, and were high in carbohydrate with decreasing protein. These changes resemble those found in blighted plants, though the magnitude of the changes is much greater in the case of blight.

On the other hand, MCKAY and DYKSTRA (**5**) state that there is a causal relationship between the curly top disease of beets, and blight of tomato. It is true that the regional and seasonal occurrence of the two diseases show considerable parallelism. Furthermore, in the original publication of BALL (**2**) on curly top of beet are found statements indicating light-relationships for that disease similar to those which have been observed in tomato blight.

The chemical changes connected with curly top of beet were studied by BUNZEL (**4**). Though he reports many analyses, there are few strictly comparable data in his report, and BUNZEL made no attempt to draw conclusions therefrom. However, it seems that in general, in the leaves of curly top beets, there is a reduction in sugar content and no effect upon the nitrogen. In curly top roots there is a reduction in sugar, while total and insoluble nitrogen increases. Except for the last mentioned item, the changes in curly top beets seem to be the opposite of those found in blighted tomatoes. Of course this is not absolute evidence against the theory of a common causal agency in the two diseases.

BREWER, KENDRICK and GARDNER (**3**) have studied the changes in the tomato plant accompanying the two virus diseases, mosaic and streak. These workers analyzed composite samples of the whole plant. They found that mosaic and streak infection led to reduction in per cent. of dry matter and of carbohydrates, and unchanged total nitrogen. All these changes are different from those found in the case of Western Yellow Blight.

## Conclusions

Definite changes in chemical composition characterize the disease of tomato called Western Yellow Blight. As all parts of the plant show these changes, it appears that the disease involves the whole plant.

Carbohydrates accumulate progressively in all parts of the blighted plants. It is suggested that this accumulation is the result of inability to utilize the carbohydrates, vegetative growth having been stopped by some unknown cause. The carbohydrate accumulation is no greater than it is in plants stunted through nitrogen starvation or "hardened" by exposure to low temperature, hence it cannot be considered the primary cause of growth cessation or of blight.

The total nitrogen content decreases in the leaves but increases in other parts of the blighted plant. Protein nitrogen was not determined as such,

but can be assumed to decrease, as the per cent. of nitrogen in soluble form increased in all parts of blighted plants. Nitrogen starvation can not be the cause of blight, but inability to translocate nitrogen within the plant, and inability to synthesize higher nitrogen compounds, may be more directly connected with the inception of blight.

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# THE EFFECT OF ULTRAVIOLET RADIATION UPON YEAST CULTURE MEDIA

J. W. Woodrow, A. C. Bailey and E. I. Fulmer

(WITH ONE FIGURE)

While a great deal of work has been done on the effect of ultraviolet radiation upon the growth of yeast and other microorganisms, the effect of the rays upon the culture medium itself, so far as its growth-promoting properties are concerned, has not been adequately studied. It is obvious that the effect of the ultraviolet rays upon the organism in the medium may be due to a combination of factors, the direct effect upon the organism and an indirect action due to changes in the composition of the medium. This communication presents data on the effect of ultraviolet radiations upon the ability of several culture media to support the growth of yeast.

## Equipment and methods

The source of the rays was a Cooper-Hewitt quartz lamp operated on a direct current of 110 volts and 4 amperes. Wratten filters were used in some cases to control the range of radiations used, and clear quartz "Vitreosil" Erlenmeyer flasks and Pyrex flasks were used as culture vessels.

The culture of *Saccharomyces cerevisiae* had been growing continuously for a period of years upon synthetic media. The counts were made with the Thoma-Zeiss chamber. When the count is one there are 250,000 cells per cubic centimeter. All incubations were made at 30° C.

The intensity of the ultraviolet was measured in lithopone units according to Clark (**16**). This unit is defined as the energy required to darken the lithopone paste to a reflection factor of 50. Exposures of the media were made at 16 cm., under which condition six seconds were required to produce the standard darkening.

The media used were C, D and E as developed and described by Fulmer, Nelson and Sherwood (**18**). The composition of these media, in terms of grams of solute per 100 cubic centimeters of medium, is shown in table I.

Each flask was weighed with contents before inoculation so that any evaporation during sterilization or irradiation could be compensated by the addition of the required amount of sterile water. In each instance 25 cc. of the medium were used in a 50 cc. quartz or Pyrex flask. The flasks were tipped at an angle so as to receive a maximum amount of the radiant energy.

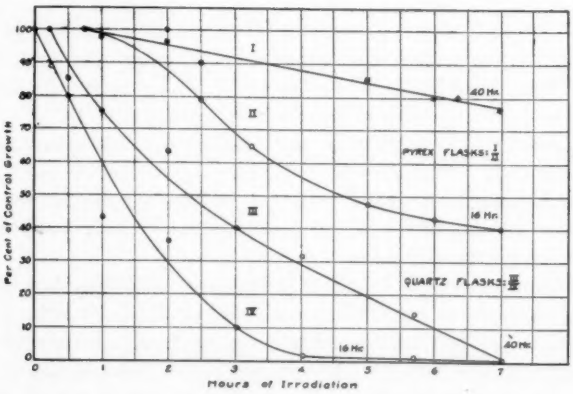
TABLE I

COMPOSITION OF YEAST MEDIA USED

| CONSTITUENTS | MEDIUM | | |
|---|---|---|---|
| | C | D | E |
| | gm. | gm. | gm. |
| $NH_4Cl$ | 0.188 | 0.188 | 0.188 |
| $K_2HPO_4$ | 0.100 | 0.100 | 0.100 |
| $CaCl_2$ | ..... | 0.100 | 0.100 |
| $CaCO_3$ | ..... | ..... | 0.040 |
| Sucrose | 10.000 | 10.000 | 10.000 |

## Experimental results

The results obtained for medium E irradiated for various time intervals are shown in figure 1. For comparative purposes the growth in the non-irradiated control flask has been taken as one hundred. These results show that with both the quartz and Pyrex flasks irradiation causes the development of toxicity in the medium, the effect being much greater for the quartz. These results are typical of many experiments. Irradiation of media C and D gave the same general results both for quartz and Pyrex flasks as those for medium E. Evidently the presence of the calcium chloride or carbonate does not influence results.




In order to determine whether heat rays had any part in the development of the toxicity in the medium these rays were filtered out by about 4 cm. of slowly running water. The receptacle containing the water was 6.25 x 12.5 cm. by 6.25 cm. deep. A quartz plate 2 mm. thick served as the

bottom of this filter, which allowed the ultraviolet radiations and the visible light rays to pass on through. Beneath this filter was placed a screen with an opening 5 x 5 cm. Across this opening were placed quartz, pyrex, and Wratten light filters, the results with the Wratten filters being tabulated in table II. These filters were ten centimeters from the lamp, and the flasks were ten centimeters from the filters. The energy reaching the flasks must be considerably less than it would be without the filter system. Nevertheless, even the short time irradiations, with the infra red rays filtered out, gave reduced counts equivalent to counts resulting from much longer irradiations when these rays were present.

In table II data are presented showing the growth of yeast in medium E exposed in quartz flasks for 30 minutes through Wratten filters under the conditions which have been outlined.

## TABLE II

WRATTEN FILTERS, 30 MINUTES IRRADIATION

| FILTER NO. | WAVE LENGTH AT MAXIMUM INTENSITY | RANGE OF FILTER | COUNT |
|---|---|---|---|
| | mμ | mμ | 20 hours |
| 17 | 350 | 300–425 | 7.6 |
| 34 | 420 | 315–500 | 5.8 |
| 47 | 440 | 350–530 | 16.0 |
| 40 | 500 | 450–630 | 21.6 |
| Control | ..... | ..... | 24.6 |

It is apparent that the toxic effect of irradiation is more severe with the shorter wave lengths, the effect being small for lengths longer than 440–500 mμ.

The data plotted in figure 1 show that after 40 hours of incubation the toxic effect in some cases is not so evident as after the 16-hour period. This may be due to the acclimatization of the yeast or to the disappearance of the toxic agent on standing. To test the latter point, flasks were irradiated for various lengths of time and either allowed to stand for various periods up to one week or were vigorously boiled. In neither case did the toxicity diminish to an appreciable extent. Evidently the toxic agent is non-volatile.

By irradiating separately distilled water, a solution of the salts, and the sugar solution, it was evident that the toxicity was entirely due to the effect of the ultra-violet rays upon the sugar. Similar toxicity developed by the irradiation of solutions of dextrose, calcium gluconate, or glycerol. Irradiated air with or without the removal of the ozone did not cause the development of toxicity nor was the toxicity a pH effect.

The effect of ultra-violet light upon the carbohydrates in solution has been studied by BERTHELOT and GAUDECHON (**7, 9**), BIERRY, HENRI and RANC (**14, 15**), BIERRY and HENRI (**12, 11**), and RANC (**20**). The following decomposition products are reported on long exposure (40–70 hrs.): $H_2$, CO, $CO_2$, $CH_4$, HCHO. Sucrose is inverted and an acidity develops. BIERRY, HENRI and RANC (**13**) report that the exposure of glycerol to the ultra-violet leads to the formation of β-acrose in an alkaline medium. The reaction of other organic compounds has been studied by BERTHELOT (**1**), BERTHELOT and GAUDECHON (**2, 3, 4, 5, 6, 8, 10**), EULER and LINDBERG (**17**), and LOMBARD (**19**).

Although it has been shown here that the toxic agent is non-volatile it seemed advisable to study the action of CO on the medium. The gas was bubbled through the medium for various lengths of time. The one hour and three hour treatment cut the growth to 75 per cent. and 60 per cent. of the control, a toxicity not comparable with the irradiation. Moreover the toxicity disappeared on boiling. Evidently the toxic material is not carbon monoxide nor the result of the action of that substance upon the medium.

## Summary

Upon exposure to ultra-violet light the yeast media developed a toxicity which increased with the length of time of irradiation. The toxic factor is non-volatile. The effect of ultra-violet upon yeast in these media must be due in part at least to alteration in the composition of the medium and not entirely to the direct effect upon the organism. In these experiments the toxic factor results from the action of the short rays on the sugar in the medium. For the study of the direct action upon the cell, a medium must be used whose growth-promoting properties are not changed by the ray treatment.

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# SEMIPERMEABILITY OF SEED COVERINGS AND STIMULATION OF SEEDS*

FELIX KOTOWSKI

In a previous paper (**6**) the writer assumed that the methods of seed stimulation, advocated by POPOFF (**9**) are of little value for agriculture and horticulture [*cf.* also BREDEMAN (**1**)]. Many publications witness the great interest on the subject of seed stimulation. The occurrence of stimulation itself and the effect of so-called stimulants upon yield is still in controversy. However, the search for principles involved has been meager. These investigations aim to contribute to the knowledge of one of these principles, which is not the least important in the writer's opinion. The well established fact of semipermeability of seed coverings was overlooked by POPOFF and his associates. In this paper the term seed coverings is used to include all structures outside the embryo and endosperm. The significance of the semipermeability of the seed coats was stated by SHULL (**11**, p. 191): "The testa has physical and chemical characters, which may enable it to modify greatly any factors entering into germination behavior and the effects of these characters must be known before any sound conclusions can be drawn. Semipermeability is now shown to be common enough that its existence should be proved or disproved before proceeding to use stimuli acting through membranes." This was written ten years before POPOFF started to publish his papers on the topic of seed stimulation.

POPOFF (**9**) claimed to explain the real cause of stimulation by reagents. Unfortunately, he and his associates when presenting successful results did not give the exact composition of the stimulants used and therefore their work is not susceptible of verification, but it is certain that they have paid no attention to the permeability of the seed coverings.

## Methods

Samples of seeds (50–80 gm.) were washed in tap water five minutes and rinsed two minutes in running distilled water. In this way, probably all salts on the surface of the seeds were removed. After washing, the seeds were dried 12 hours at 40° C. and used 12 hours later. Two and one half gram samples of seed were soaked 16 hours in 25 cc. of the solutions

* Work done at Davis, Calif., Division of Truck Crops, University of California, while on leave from College of Agriculture, Warsaw, Poland.

used, in 100 cc. flasks. The checks were similarly soaked in distilled water, after which the samples were divided into two groups.

In one group, the seeds were separated from the liquid, dried and weighed, and the gain of weight calculated as percentage of the air-dry weight. A uniform procedure was adopted for this drying. The seeds were blotted with filter paper for one minute and then later dried with a cloth for one minute. After weighing, the seeds were immediately soaked in 25 cc. of distilled water for 4 hours and were again dried and weighed. In the second group of samples, the seeds after being soaked 16 hours in solutions or in distilled water were removed from the liquid and rinsed in running distilled water for one minute; the further proceedings were the same as in the first group. The electrical conductivity of the solutions, and of the distilled water in which the seeds were immersed, was determined as soon as the soaking stopped.

The standard deviation of the weights was 2.5 per cent. of the mean, working with 4 replications of each kind of seed and for each solution.

The intake of water was almost complete after 16 hours; the gain during the next 4 hours was very small (0.03 cc. average), therefore the calculations have been made on the basis of the 16-hour water intake.

The intake of salts by the seeds was determined by the measurement of electrical conductivity of the external solutions. This method shows minute differences in concentration of aqueous salt solutions and detects small amounts of salts accurately and promptly. Water of high degree of purity was used ($C = 2.8 \times 10^{-6}$ mhos) and the measurements and computations were made according to FINDLAY (**4**).

Since the rate of water and salt intake and the processes of diffusion are influenced by temperature, the experiments were run at $20 \pm 1°$ C. The conductivity measurements also were made at $20 \pm 0.1°$ C. by means of KOHLRAUSCH's method. A dipping electrode was used in the solutions.

STILES (**12, 13, 14**) used conductivity determinations in the study of permeability of plant tissues, but so far as the writer is aware this method has not been used in permeability experiments with seeds. The results obtained by the conductivity method may need revision before conclusions can be drawn.

The corrections to be made were reported by STILES and KIDD (**12**) and in so far as they can refer to seeds, may be briefly stated. The possible causes making for a fall of conductivity in the external salt solutions aside from absorption, and which therefore make the values obtained greater than the true values for absorption, are as follows: 1. Reactions between the exudate and the external solutions by which non-ionized molecules are produced. 2. An increased exosmosis due to the action of the salts on the

tissues, as compared with this phenomenon, is distilled water. 3. An independent absorption of ions (*cf.* also PANTANELLI, **8**).

These experiments were affected with these three sources of error, but estimation of any of them is impossible, although in the case of seeds, most important is the diffusion of carbon dioxide evolved by respiration. The check series (soaked in distilled water) aimed to estimate this error, but it was merely assumed that the respiration process was equal in distilled water and in solutions, which may not be entirely true.

The value for intake or for diffusion of salt was found from the equation: $C_2 = C_2 - (C_1 + C_x)$; in which $C_2$ is the final conductivity of the solution, $C_1$ the initial conductivity of the solution, $C_x$ the increased conductivity caused by life processes of seeds, determined in check series; for distilled water $C_1 = 0$.

The diffusion of salts from seeds soaked and washed for one minute as well as from soaked and unwashed seeds was compared. The diffusion from washed seeds was lower, and the difference was considered as the loss due to washing for one minute. This loss represented the minimum quantity of salt that did not penetrate the seed coverings. The technique used for this purpose was very gentle indeed when compared to that of WOLFE (**16**), who says that LiCl (saturated solution) was only superficially absorbed by barley grains, however, since it could all be removed by an *hour's* washing in running water.

Two salts, the kations and anions of which are claimed by POPOFF to be good stimulants, were chosen. $KNO_3$ and $MnSO_4 \cdot 4H_2O$ in 1.50 per cent. aqueous solutions were used for soaking the seeds. Their conductivity at 20° C. was as follows: For 1.5 per cent. (= 0.15 N) $KNO_3$, $C_1 = 33.80 \times 10^{-3}$ mhos; for 1.5 per cent. (= 0.067 N.) $MnSO_4 \cdot 4H_2O$, $C_1 = 13.20 \times 10^{-3}$ mhos.

Viable seeds of *Spinacia oleracea inermis, Cucumis sativus, Capsicum annuum, Triticum vulgare, Secale cereale* and of *Hordeum distichum* were tested. The seeds were carefully selected to eliminate injured ones, but in cereals besides the selected (A) grains, non-selected (B) samples of commercial seed were tested. The examination of the latter showed 20 to 30 per cent. of grains more or less injured in barley and rye, and 5 per cent. in wheat.

## Results

### ABSORPTION AND PERMEABILITY FOR $KNO_3$

The absorption of $KNO_3$ and water by these seeds, and the loss of salt by exosmosis from seeds of the various plants used, are shown in table I.

The highest amount of salt was taken in by spinach and pepper (2.1 per cent. of the weight of seeds); lower was the gain for wheat A and B, rye B

## TABLE I

SAMPLES 2.5 GM. SOAKED IN 1.5 PER CENT. $KNO_3$ (=0.15 N) AT 20° C. FOR 16 HOURS

| CHANGES OBSERVED | SPINACH | CUCUMBER | PEPPER | WHEAT A | WHEAT B | RYE A | RYE B | BARLEY A | BARLEY B |
|---|---|---|---|---|---|---|---|---|---|
| Salt absorbed, mg. | 50.90 | 10.40 | 50.80 | 25.00 | 24.30 | 11.70 | 25.00 | 8.50 | 27.10 |
| Water absorbed, cc. | 2.30 | 1.22 | 2.34 | 1.02 | 1.23 | 1.40 | 1.46 | 1.00 | 1.08 |
| Concentration of salt in seed, per cent. | 2.21 | 0.85 | 2.16 | 2.45 | 1.98 | 0.84 | 1.71 | 0.87 | 2.49 |
| Loss of absorbed salt due to exosmosis during 4 hours, seeds unwashed, as percentage of amount absorbed | 60.00 | 76.50 | 80.00 | 54.50 | 35.00 | 54.00 | 33.00 | 78.00 | 29.00 |
| Loss of absorbed salt due to exosmosis during 4 hours, seeds washed, as percentage of the amount absorbed | 15.70 | 24.00 | 13.80 | 9.00 | 18.80 | 31.50 | 17.40 | 24.40 | 13.00 |
| Loss of absorbed salt due to the washing of seeds one minute, as percentage of the amount absorbed | 44.30 | 52.50 | 66.20 | 45.50 | 16.20 | 22.50 | 15.60 | 53.60 | 16.00 |

and barley B (1 per cent. of the weight of seeds); the intake of $KNO_3$ by cucumber, rye A and barley A is very moderate (0.4 per cent. of the weight of seeds). The intake of salt is in some degree dependent upon the intake of water. This fact is shown by the values for the final concentration of the salt within the seeds after 16 hours of soaking. On the basis of these figures we can classify the seeds as follows: First, barley B and wheat A, having final inside concentration of 2.47 per cent. compared with a final external concentration of 1.45 per cent; then pepper, spinach, wheat B and rye B, with an inside concentration of 2.01 per cent. against 1.45 per cent. outside concentration. All these seeds possessed higher concentration of $KNO_3$ within the seeds than outside, when the soaking was stopped. A reverse case could be noticed in cucumber, rye A and barley A; for these seeds the final inside concentration of salt was 0.85 per cent. against 1.53 per cent. of the external solution.

It is very obvious that the uninjured grains of barley and rye showed slight intake of $KNO_3$ in comparison with the injured ones. This was not the case with wheat, for which, however, SCHROEDER (**10**) found impermeability of seed coverings against $KNO_3$.

The behavior of whole grains of barley and rye as well as those of cucumber leads to the conclusion that the seed coverings checked the intake of salt and its passage into the tissues of the seed within the layers of the seed coverings.

As far as wheat, pepper and spinach are concerned, it is rather difficult to explain their greater intake of $KNO_3$ as caused by permeability of seed coverings. It seems more justifiable to credit this to the layers of testa and pericarp in which the salt was accumulated. Here we meet a problem that is very important in connection with the methods of seed stimulation used by POPOFF, and an attempt was made to determine the localization of the salt taken in by the seeds.

To secure some reliable data, studies of the leaching effect of distilled water were made. The seeds were removed from the salt solution, dried, and soaked four hours in distilled water. This represented only one fourth of the time spent in the salt solution, but diffusion was great enough to be detected by conductivity measurements. The losses due to this process were calculated in percentage of the salt taken up by the seeds (*cf.* table I). They were different for seeds previously washed and for unwashed seeds. We will first consider the unwashed seeds.

The relation between the intake of salt and the ease of its removal from the seeds is plain. When the ions of $KNO_3$ penetrated the embryonic seed tissues, as was the case in samples B of the cereals, the exosmosis was small, and dropped to about half of that loss which was shown by the samples A

(32.3 versus 62.4 per cent.). The vegetable seeds, being all carefully selected, showed losses even greater than those of cereals A, *i.e.*, 72.2 per cent., which indicates that their seed coverings were chiefly responsible for absorption of $KNO_3$, although the salt was accumulated in a higher degree than by cereals A.

This is corroborated by the behavior of washed seeds. The rinsing of seeds was exceedingly short (about 0.001 of the time for the intake of salt), nevertheless, it was possible to get distinct differences in diffusion, when washing took place.

After washing, during the same time and in the same conditions, diffusion was decreased for spinach, cucumber and pepper from 72.2 to 17.8 per cent. (loss due to washing 54.4 per cent. of the intake of salt); for cereals A from 62.4 to 19.3 per cent. (loss due to washing 43.1 per cent.); and for cereals B from 32.3 to 16.4 per cent. (loss due to washing being only 15.9 per cent. of the intake of salt). The low numbers for cereals B are in good agreement with our assumption that $KNO_3$ was really absorbed by grains with injured seed coverings and therefore elimination of the salt from the tissues was not possible by short washing. In the other groups in which intact seed coverings prevented the penetration of $KNO_3$ to the endosperm and embryo, the salt was easily removed, indicating the superficial character of its intake. Accordingly, there is little probability, if any, that in vegetable seeds and in cereals A the kation and anion could act on the protoplasm and stimulate growth (*cf.* also KOTOWSKI, **6**).

### ABSORPTION AND PERMEABILITY FOR $MnSO_4$

The absorption of $MnSO_4$ and water, and exosmotic loss of salts from the seeds used, are shown in table II.

The seeds of spinach and pepper showed the largest quantities of salt taken in (2.1 per cent. of the seed weight). The gain in salt by barley A and B and wheat B was one half of this (1.1 per cent. of seed weight) and the minimum amount was absorbed by rye A and B and cucumber (0.8 per cent. of seed weight). For barley A and B, the final inside concentration was 2.91 against 1.43 per cent. final outside concentration. A lower gradient was found in spinach, pepper, and wheat A and B (2.22 versus 1.44 per cent.). The external solution was stronger than the internal only for rye A and B and for cucumber (1.38 versus 1.5 per cent.). The seeds of vegetables were similar in their intake of $MnSO_4$ and $KNO_3$.

In the case of cereals, the difference between samples A and B was small. The intact seed coverings checked but little the intake of $MnSO_4$, which in quantity exceeded $KNO_3$. Each cereal seemed to exhibit a specific behavior toward the intake of $MnSO_4$, apparently with little regard to the seed coverings.

TABLE II

SAMPLES 2.5 GM. SOAKED IN 1.5 PER CENT. $MnSO_4 \cdot 4H_2O$ (= 0.067 N) AT 20° C., 16 HOURS

| CHANGES OBSERVED | SPINACH | CUCUMBER | PEPPER | WHEAT A | WHEAT B | RYE A | RYE B | BARLEY A | BARLEY B |
|---|---|---|---|---|---|---|---|---|---|
| Salt absorbed, mg. | 53.10 | 18.70 | 52.50 | 21.60 | 27.50 | 16.20 | 21.00 | 26.80 | 33.40 |
| Water absorbed, cc. | 2.30 | 1.22 | 2.34 | 1.02 | 1.23 | 1.40 | 1.46 | 1.00 | 1.08 |
| Concentration of salt in seed, per cent. | 2.30 | 1.54 | 2.24 | 2.12 | 2.24 | 1.16 | 1.44 | 2.72 | 3.10 |
| Loss of absorbed salt, due to exosmosis during 4 hours, seeds non-washed, as percentage of the amount absorbed | 39.00 | 34.60 | 57.50 | 43.50 | 18.70 | 37.00 | 35.00 | 21.00 | 21.70 |
| Loss of absorbed salt, due to exosmosis during 4 hours, seeds washed, as percentage of the amount absorbed | 9.00 | 12.60 | 19.20 | 12.90 | 7.50 | 15.20 | 15.00 | 13.20 | 9.60 |
| Loss of absorbed salt, due to the washing of seeds one minute, as percentage of the amount absorbed | 30.00 | 22.00 | 38.30 | 30.60 | 11.20 | 21.80 | 20.00 | 7.80 | 12.10 |

STILES and KIDD (**14**) reported that bivalent ions are at final equilibrium absorbed to a much less extent than the monovalent ions. In their experiments the tissues of carrot were soaked for 42, 64.5, 71.5 and 91 hours. It can be concluded in the present investigation that the 16-hour period of soaking was far from equilibrium, as to the salt, and one can apply rather the initial order of the rate of absorption, in which first place is always given to K. Anions appeared, according to STILES and KIDD (**14**), to be absorbed most rapidly at first in the order: $SO_4$, $NO_3$, Cl, but later in the order: $NO_3$, Cl, $SO_4$. This observation fits with our case, as far as the cereals are concerned, if we suppose that $SO_4$ was the ion more absorbed from $MnSO_4$ and K from $KNO_3$. It was not possible to determine which properties govern the rate of intake of $MnSO_4$ in cereals, and it would be premature at present to make a suggestion.

Considering now the losses due to diffusion of salt from unwashed seed, we record for vegetable seeds 43.7 per cent., for cereals A 33.8 per cent., and for cereals B 25.1 per cent. loss.

The judgment upon the character of the intake of $MnSO_4$ was supported by examination of the diffusion from seeds washed for one minute. A decrease was found, however, smaller than for $KNO_3$. The vegetable seed showed a diminution from 43.7 to 13.6 per cent., and the loss of salt due to washing was 30.1 per cent.; for cereals A, the decrease was from 33.8 to 13.8 per cent., the loss due to washing being 20 per cent.; for cereals B the diffusion changed from 25.1 to 10.7 per cent., the loss due to washing being 14.4 per cent. The percentages obtained in this case were all lower than those found for the diffusion process when $KNO_3$ was used. These results indicate that these two salts act in some ways differently on the seeds in question.

The ions of $MnSO_4$ entered into the seeds in larger quantities and moreover were not so easily withdrawn as the ions of $KNO_3$. This may be seen from the following comparison. If the losses due to diffusion from unwashed vegetable seeds in case of $MnSO_4 = 1$, then we have for $KNO_3$ losses $= 1.6$; the washing process produces a loss of $MnSO_4 = 1$ as against 1.8 for $KNO_3$. In the samples of cereals with intact seed coverings only, the ratios for unwashed seeds are 1 : 1.8, and for washed seeds are 1 : 2.1, respectively. But little difference occurred within the samples of cereal grains with some seed coverings injured. The amount of eliminated salt from unwashed seeds with injured seed coverings was for $MnSO_4 = 1$ and for $KNO_3 = 1.3$, and the losses due to washing were as 1 ($MnSO_4$) : 1.1 ($KNO_3$). In the last group it is clear that the washing process exercised the same effect on the seeds soaked in solutions of both of the salts. From this presentation of ratios we can say that the limiting factor for the penetration of $KNO_3$

and $MnSO_4$ was the impermeability of seed coverings. When this factor was not operative the salts reached the embryo protoplasm and there was a real absorption by tissues other than testa and pericarp.

The results of these experiments indicate the important rôle played by the seed coverings in questions of seed stimulation.

GOLA (**5**) was the first to demonstrate and announce the existence of a selective semipermeable layer in seed coverings, and in an examination of the seeds of 500 species, belonging to some 40 families, such membranes were found to be regularly present, except in the *Leguminosae* and in certain genera of the *Cistaceae* and *Cruciferae*. BROWN (**2, 3**) made the first quantitative study of the phenomenon, especially on barley seeds, and various other workers contributed extended quantitative studies made on other seeds; notably MAREL (**7**) on squash, SCHROEDER (**10**) on wheat, SHULL (**11**) on cocklebur; TJEBBES (**15**) claimed that seeds of the sugar beet probably have a selectively permeable membrane as a part of the inner seed coat.

The papers of BROWN, MAREL, and SCHROEDER deal with solutions of high concentration. But the permeability of seed coverings to the dilute solutions of salts applied as stimulants has never been proved to the writer's knowledge.

## Summary

The effect of 16 hours' soaking in 1.50 per cent. solutions of $KNO_3$ and $MnSO_4 \cdot 4H_2O$ was studied for 9 lots of seeds. The intake of the salts by the seeds, and the diffusion of the salts from the seeds in subsequent soaking, was determined by means of electrical conductivity measurements of the external solutions. From the data recorded, the following conclusions may be drawn:

1. The intake of salt depends on the kind of seed and on the kation and anion of the salt, but the limiting factor is apparently the seed coverings.

2. The seed coverings exercised a distinct check in the intake of $KNO_3$, especially by cereals.

3. When the seed coverings were intact, absorbed salts were held superficially. Accordingly, heavy losses in the amount of salts absorbed resulted when the seeds were washed one minute.

4. The samples of cereals with grains partially injured showed high intake of salt and slight losses due to washing, owing to the permeability of spots deprived of seed coverings.

5. The behavior of seed coverings in the presence of chemical stimulants must be known in order to escape failures with seed stimulation in work done on larger scale.

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# THE USE OF ETHYLENE, PROPYLENE, AND SIMILAR COMPOUNDS IN BREAKING THE REST PERIOD OF TUBERS, BULBS, CUTTINGS, AND SEEDS

G. A. VACHA AND R. B. HARVEY

(WITH ONE PLATE)

It has been known for some time that ether (**8, 4, 9**), chloroform, ethyl bromide (**1, 5**), and other substances (**6**) may be used for breaking the rest period of certain plant materials. Since the time of undertaking the study here reported, the use of ethylene for a similar purpose has been advocated by ROSA (**7**), and DENNY (**2, 3**) has published upon the applications of ethylene chlorohydrin, thiourea, potassium thiocyanate, and other substances in breaking the dormancy of potatoes. DENNY did not find ethylene or propylene especially effective in breaking the dormancy of potatoes. In this study trials of the comparative activity of ethylene, propylene, and ethylene oxide in breaking the rest period have been made with a number of seeds, tubers, bulbs, and cuttings.

## Breaking the dormancy of potatoes with ethylene

In making tests of potatoes for the presence of mosaic by the tuber index method it is desirable to secure as uniform germination of the samples as possible. It is desirable to know within as short a time as possible after digging, the degree of freedom of the seed samples from mosaic diseases so that they may be judged as to grade. The potato has a rest period during which it will not sprout even if placed under favorable conditions.

Selected tubers of Early Ohio, Rural New Yorker, Bliss Triumph, Burbank Russet, Green Mountain, and Irish Cobbler were obtained from A. G. TOLAAS, in charge of the Minnesota Seed Potato Certification. The samples of these varieties were kept uniform in growth in the field and in storage conditions preliminary to trial.

The tubers were divided into four lots, and six tubers of each variety were selected for each lot, or, in other words, each lot contained six varieties of potatoes and six tubers of each variety. The tubers were carefully selected so as to have the lots as uniform in size as possible. Only those tubers free from mechanical injury and disease were used.

Lots 1 and 2 were then put into culture ovens where the temperature was maintained at 20° C. A beaker full of $H_2O$ was set inside each oven to keep the air moist.

Lot 1 was then treated with ethylene gas, one part to one thousand by volume, while lot 2 was used as a check. At the end of thirty-six hours lot 1 received the second dose of ethylene. At the end of seventy-two hours the third dose was applied, and a fourth dose after 120 hours. The total length of treatment was six days.

A cylinder of ethylene oxide was cooled to 0° C. to liquefy it, and an aqueous solution of 1 cc. of ethylene oxide to 1,000 cc. of water was prepared. Lot 3 was soaked in this solution for twenty-four hours. Lot 4 was soaked for twenty-four hours in water. Both lots were held at room temperature.

At the end of the treatment the tubers were spread out and dried quickly by blowing a stream of warm air over the tubers. After drying, each lot was cut by means of a cork borer into pieces three-fourths of an inch in diameter. This gave a very uniform sized seed piece having an average weight of one-fourth ounce. The seed pieces were allowed to dry for two hours before planting.

Thirty seed pieces were then selected from each variety and planted in a cutting bench in the greenhouse. The seed pieces were planted in rows eight inches apart, and four inches apart within the row. Depth of planting was two inches. The soil used for planting was of the 3–2–1 mixture, light clay, sand, and leaf mold. All four lots received bottom heat, the same amount of light, and about the same quantity of water. Data were first recorded when the sprouts appeared above the surface of the ground, and at other intervals. Table I indicates the marked difference in the length of the dormancy among the various varieties as shown by the number sprouting at fifteen and forty-eight days. Evidently, the Early Ohio variety has the longest period of dormancy, followed by Rural New Yorker, Green

TABLE I

EFFECT OF ETHYLENE ON SPROUTING OF POTATO VARIETIES

| VARIETY | NUMBER APPEARING ABOVE GROUND AFTER PLANTING[1] | | | |
|---|---|---|---|---|
| | 15 days | | 48 days | |
| | Ethylene Lot 1[2] | Check Lot 2[3] | Ethylene Lot 1[2] | Check Lot 2[3] |
| Early Ohio | 0 | 0 | 27 | 23 |
| Rural New Yorker | 2 | 0 | 28 | 25 |
| Bliss Triumph | 18 | 3 | 30 | 29 |
| Burbank Russet | 19 | 4 | 30 | 30 |
| Green Mountain | 4 | 1 | 29 | 29 |
| Irish Cobbler | 8 | 4 | 30 | 30 |

[1] 30 seed pieces of each variety were planted.

[2] Treated with ethylene 1: 1,000.

[3] Check kept in air six days.

Mountain, and Irish Cobbler. The majority of the seed pieces which received the ethylene treatment had sprouted in twenty days, except the Early Ohio. The majority of these appeared above ground in twenty-eight days after planting. In the case of lot 2 (check) a longer time was required to obtain complete sprouting, namely thirty-five days. This would indicate that the ethylene treatment speeded sprouting from seven to fifteen days.

In lot 4 the tubers soaked in $H_2O$ for twenty-four hours required thirty-five days for complete sprouting. Hence soaking the tubers in water had no effect on dormancy. Of the tubers in lot 3 soaked in a water solution of ethylene oxide (1:1,000), only few sprouted, which indicates that ethylene oxide is very toxic. Most of the seed pieces were decayed when dug up.

In each case the ethylene-treated seed pieces, when once above the ground, grew much faster than the untreated ones. This is very clearly demonstrated by the photographs, figures 1, 2, 3, 4, 5, Plate I. The great stimulation in all of the tubers treated with ethylene is noticeable. Only one set treated with ethylene oxide is shown (table II), because most of

## TABLE II

EFFECT OF ETHYLENE OXIDE ON SPROUTING OF POTATO SEED PIECES

| VARIETY | NUMBER APPEARING ABOVE GROUND AFTER PLANTING[1] | | | |
|---|---|---|---|---|
| | 15 days | | 48 days | |
| | Ethylene oxide Lot 3[2] | Check Lot 4[3] | Ethylene oxide Lot 3[2] | Check Lot 4[3] |
| Early Ohio | 0 | 0 | 0 | 25 |
| Rural New Yorker | 1 | 1 | 1 | 26 |
| Bliss Triumph | 2 | 2 | 2 | 30 |
| Burbank Russet | 3 | 1 | 4 | 30 |
| Green Mountain | 0 | 1 | 2 | 26 |
| Irish Cobbler | 0 | 4 | 0 | 28 |

[1] 30 seed pieces of each variety were planted.

[2] Treated with ethylene oxide 1: 1,000 in water for 24 hours.

[3] Check immersed in water 24 hours.

the seed pieces rotted. Evidently this ethylene treatment will offer considerable advantage in obtaining rapid growth for the determination of tuber index for the mosaic diseases.

### Treatment of corms and bulbs to break the rest period

The growing of gladiolus in greenhouses during the winter months is not a profitable business on account of the long time required for germina-

tion. For this reason only a limited amount of gladiolus is grown under glass. Commercial florists have expressed a desire for growing more gladiolus under glass if a way to treat them to shorten the length of the rest period could be found.

Forty corms of gladiolus were selected and arranged into five lots; twenty corms to lot 1 and five corms to each of the other four. The corms were carefully selected so as to be free from disease and injury, and they were as uniform in size and weight as possible. All the corms were put into air-tight glass-stoppered bottles for treatment as follows:

Lot 1. Kept in air.
Lot 2. 2 cc. of ethyl ether were added to 100 cu. inches air space.
Lot 3. 2 cc. of chloroform were added to 100 cu. inches air space.
Lot 4. Received ethylene gas, one part to one thousand parts of air.
Lot 5. Received propylene gas, one part to one thousand parts of air.

The six jars were put into a culture oven where the temperature was maintained at 20° C. After six days the corms were removed from their respective jars and planted in flats of soil.

TABLE III

EFFECT OF TREATMENT WITH ANAESTHETICS UPON SPROUTING OF GLADIOLUS

| LOT | TREATMENT | NO. OF CORMS TREATED | NO. GROWING 32 DAYS AFTER PLANTING |
|---|---|---|---|
| 1 | Check | 20 | 1 |
| 2 | Ether | 5 | 5 |
| 3 | Chloroform | 5 | 4 |
| 4 | Ethylene | 5 | 4 |
| 5 | Propylene | 5 | 3 |

As shown in table III, most of the treated corms were growing, while only one of the checks was above ground thirty-two days after planting. This experiment was repeated with a larger quantity of corms. Practically the same results were obtained. On account of the difficulty of getting larger numbers of corms of the same variety which were exactly of the same maturity, only the trial with perfectly uniform corms is reported. These experiments indicate that the dormant period of gladiolus corms was reduced by one half. It is estimated that the growth was advanced twenty-five to thirty days.

It is interesting to note that at the concentrations used ether seems to be most efficient in breaking dormancy, followed by chloroform. No differ-

ence was noted between the ethylene- and propylene-treated corms. Evidently these two homologues are about equally effective. However, the corms which received the ethylene and propylene treatment seem to grow faster than those treated with ether and chloroform, probably on account of some injury by these latter substances.

In later experiments on the larger lots the average height was determined, giving for ether- and chloroform-treated 5½ inches while the plants treated with ethylene and propylene averaged 8¼ inches tall. Ethylene oxide, concentration 1:1,000 was also used, but it appears to be very toxic and kills the tissues.

Dahlia tubers and cuttings of cannas were treated with ethylene 1:1,000. Ethylene did not seem to hasten growth in the dahlias, but it did stimulate the development of a greater number of buds on each root of cannas. On each cutting of canna which received a dose of ethylene two to four buds developed, giving two to four plants of good vigor, while the checks with few exceptions gave only one plant per cutting.

### Effect of ethylene and propylene on hardwood cuttings

Hardwood cuttings of apple, plum, cherry, golden willow, red osier, lilac, alpine currant, grape, cottonwood, common elder, high bush cranberry, mock orange, honeysuckle, and pear were treated with ethylene and with propylene (1:1,000). Two doses were given, the second dose following forty-eight hours after the first application.

The dormancy of all of the above-mentioned twigs was broken so that leaves developed, and in the case of apple and plum flower buds also developed. There seems to be very little if any difference between ethylene and propylene in their ability to break the dormancy of buds.

### Effect of ethylene and propylene on the germination of seeds

Dormant seeds of common buckthorn, high bush cranberry, snowberry, and Tartarian honeysuckle were treated with ethylene, 1:2,000, 1:1,000, 1:500, and 1:200 in air. Also propylene was used in the same concentrations. Buckthorn seeds were soaked for two minutes in concentrated $H_2SO_4$, washed thoroughly in $H_2O$, and finally washed in a two per cent. solution of sodium carbonate. These seeds were then treated for six days with ethylene 1:500. They gave almost a perfect germination in thirty-five days. Buckthorn seeds receiving a treatment of ethylene 1:500 alone showed fifty per cent. germination of the seeds in thirty-five days; slightly less than fifty per cent. germination when a concentration of 1:1,000 was used. Ethylene or propylene 1:500 for eight days in three doses gave good germination in the case of dormant seeds of high bush cranberry,

snowberry, and Tartarian honeysuckle. The use of concentration higher than 1:500 produces some injurious effects.

## Conclusions

The dormancy of potato tubers varies in length, being longest in the case of Early Ohio and shortest in Bliss Triumph and Burbank Russet.

Ethylene at a concentration of one part of the gas to one thousand parts of air by volume breaks the rest period and hastens the sprouting of tubers. The time gained by such a treatment varies from seven days in the case of the Early Ohio variety to eight or nine days for Green Mountains and Rurals, to fifteen days for Burbank Russet and Bliss Triumph. The treated tubers grow faster than untreated ones. The growth stimulation by ethylene and by propylene is shown to be greater than the stimulation of ether and chloroform in gladiolus. Ethylene oxide, one part in one thousand parts of water, was toxic to potato tubers and to gladiolus. Ethylene, either alone or after treatments by sulphuric acid, is effective in securing germination of seeds of buckthorn, high bush cranberry, Tartarian honeysuckle, and snowberry. Ethylene and propylene were found about equally effective in breaking dormancy.

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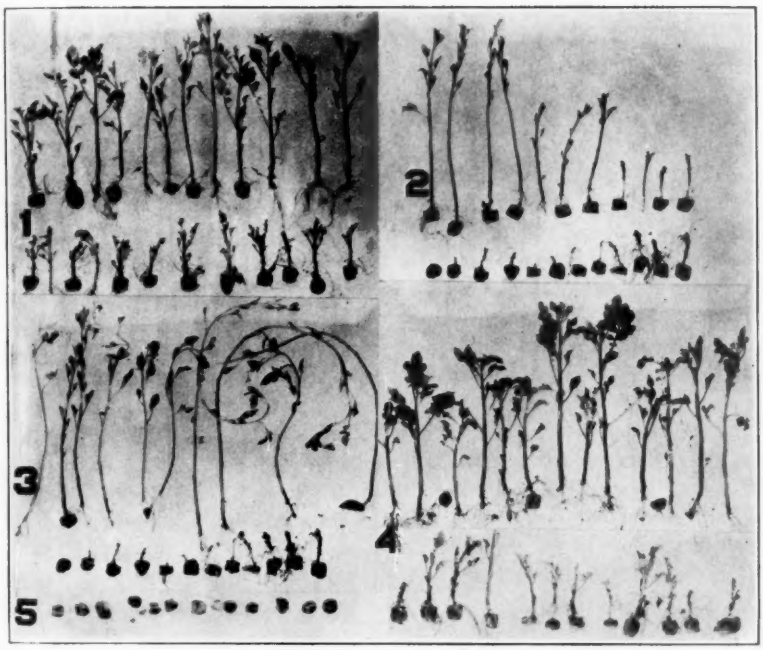


STIMULATION OF GROWTH OF POTATOES BY ETHYLENE

FIG. 1. Early Ohio. Upper row, ethylene treated; lower row, check.
FIG. 2. Green Mountain. Upper row, ethylene treated; lower row, check.
FIG. 3. Burbank Russet. Upper row, ethylene treated; lower row, check.
FIG. 4. Bliss Triumph. Upper row, ethylene treated; lower row, check.
FIG. 5. Bliss Triumph. Ethylene oxide.

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## THE DETERMINATION OF SOLUBLE CARBOHYDRATES*

It is generally agreed that soluble carbohydrates are the most available, as well as the original, source of metabolic energy for plants. Hence, studies of the formation, translocation, condensation, hydrolysis and assimilation of these compounds have commanded, and will undoubtedly continue to command, a major place in plant physiological analysis. Separation between soluble and insoluble carbohydrates is here made by classing as insoluble those compounds which require hydrolysis before they can be quantitatively extracted from plant tissues by water or alcohol. In the strictest sense the extraction of dextrins and pentosans is probably accomplished by suspension rather than by true solution, but for analytical purposes they may be termed "soluble" compounds. In the interest of completeness we have included the glucosides under this heading, although it is hardly probable that they should be classed with the carbohydrates from the standpoint of physiological function.

A brief treatment of the occurrence, solubility, reducing power and other properties of the carbohydrates here considered is given by HAAS and HILL (**4**). All but pectins and the gums are readily soluble in water. These and the polysaccharides (dextrin, inulin and mannan) are insoluble in hot 80 per cent. alcohol. It is well known that all naturally occurring free sugars with the exception of sucrose and raffinose reduce Fehling's solution. The saponins also have this property. Other glucosides, the soluble polysaccharides, pectins and gums acquire reducing power by hydrolysis, as do the non-reducing sugars. Furthermore, such reducing disaccharides as maltose and lactose acquire an increase of this property by hydrolysis. The latter carbohydrate is added to tissue in the use of commercial taka-diastase, which may contain up to 75 per cent. of the sugar.

The presence of more than two or three per cent. of pentose sugars, or of appreciable quantities of galactose or mannose, is generally considered to be an indication of poor technique in preservation or separation, since these sugars are transition compounds normally present in polymerized forms. DAVIS and DAISH (**2**) are authority for the statement that the presence of maltose or isomaltose is due to starch hydrolysis in preservation and they object on this ground to preservation by drying. Apparently germinating seedlings may be an exception to this rule, but it is general enough in its application to be of value.

* Section III of the report of the Committee on Methods of Chemical Analysis of the American Society of Plant Physiologists.

### Initial separations

Obviously, the initial separation of soluble carbohydrates depends upon a method of killing the tissue which will inhibit enzyme action. A method of extraction should then be used which will remove only those substances which can be determined together. When a limited number of substances are to be estimated it may be possible to make the initial separation with water. Generally, however, the first separation is best made with alcohol. Because of the rapidly increasing solubility of dextrin and other colloids at alcohol concentrations below 70 per cent., and the limited solubility of sugars in strong alcohol, an 80 per cent. solution of alcohol is commonly used. This extraction has the further advantage of generally separating colloidal (protein) and non-colloidal compounds of nitrogen, as well as colloidal and non-colloidal carbohydrates. The end point of the alcoholic extraction should always be determined. As a general rule, soluble carbohydrates will be removed as soon as, or sooner than, soluble nitrogen compounds and one test may thus be made for the two fractions.

A second extraction with water or 10 per cent. alcohol will remove dextrins, inulin and soluble pectins, together with a small percentage of hemicelluloses. In the absence of free fructose, acid hydrolysis may be applied to this fraction, whereas its use would be impossible if the first and second fractions were combined. This order of extraction is sometimes modified by using 50 per cent. alcohol to ensure complete extraction and then making the extract to 80 per cent. of alcohol to precipitate dextrins and other colloids.

### Carbohydrates soluble in eighty per cent. alcohol

The alcohol fraction contains the substances classed as sugars and also the glucosides. If the material has been killed by dropping it into sufficient boiling 95 per cent. alcohol to give a final concentration of 75 to 80 per cent., and extraction completed at the same strength, the colloidal carbohydrates, pectins, etc., will be practically excluded. The presence of fructose, which is claimed by Haas and Hill (**4**) to constitute more than half of the total sugars in many plants, governs the handling of this extract, and precludes the use of basic lead acetate in clearing, extended heating in acid hydrolysis, or any heating in the presence of a base or a lead salt.

Clearing.—Although 80 per cent. alcohol precipitates many interfering colloids it extracts lipoidal substances, chlorophyll, tannins, and flavones which may make filtration almost impossible when the sample is taken up in water. In addition amino-acids and tannins reduce Fehling's solution. Tannins whenever present should be removed by clearing. Unfortunately no good method is available for removing amino-acids and where these substances are present in quantities an error is introduced into the reducing

and total sugar values. This error should be small with most types of plant materials.

Three materials have been in common use as clearing reagents. Alumina cream may be used to flocculate colloids, but is of no value in removing non-colloidal impurities. Neutral lead acetate is a convenient clearing reagent which removes tannic acid, pectins and many flavones, and flocculates colloidal materials generally. The work of LOOMIS (**6**) indicates that a considerable excess of neutral lead acetate may safely be used in the presence of fructose, and also that this material answers all clearing requirements in the removal of organic impurities, when the sugars are to be estimated by copper reduction. Basic lead was shown, in the same paper, to precipitate as much as 15 per cent. of the reducing substances present, without any apparent gain in clearing effectiveness. Potassium oxalate is recommended for deleading on the basis of the work of SAWYER (**11**), MEAD and HARRIS (**8**), and LOOMIS (**7**). Hydrogen sulfide and mono-sodium phosphate remove the lead more completely than potassium oxalate, but leave the solution acid. Sodium carbonate and disodium phosphate are effective deleading agents but their alkalinity is destructive to fructose, while the use of sodium sulfate is liable to entail difficulties in filtration.

Convenience in clearing usually requires that the lead precipitate be filtered off before deleading. Otherwise the flocculating effect of the clearing reagent is lost and filtration proceeds with difficulty. Reducing impurities may also be returned to solution. As stated above, fructose must not stand with lead acetate and the solution should not be raised above room temperature, either during clearing or after deleading, unless the last traces of lead are first removed.

REDUCTION.—Two methods of copper reduction are in general use, the two minute boiling of MUNSON and WALKER (**9**), and the longer heating at 80° C. of QUISUMBING and THOMAS (**10**). Any reduction procedure must be carefully standardized and the method of QUISUMBING and THOMAS appears to have the advantage in that the temperature does not vary with altitude and barometric pressure, and variations of a few seconds in heating time constitute a much smaller percentage error with the longer heating period. It is also claimed that auto-reduction and reduction by sucrose are avoided. The MUNSON-WALKER method has the advantage of complete tables but requires more attention. However, if a simple water manometer is attached to the gas line so that the gas pressure can be adjusted accurately, the heating conditions of the MUNSON-WALKER method can be maintained without great difficulty.

A common, six-hole water bath may be used in the QUISUMBING and THOMAS method if it is provided with a motor-driven stirring device and protected from air currents. Both the bath and the burners should be sur-

rounded by an asbestos board screen. With some experience the temperature may be manually controlled with considerable accuracy by means of adjustable pinch cocks and a gas pressure manometer.

In addition to the directions on preparation of crucibles given on pp. 190–191 of the revised methods of the A. O. A. C. (**1**) it is advisable to make one or more blank runs with new crucibles until their weight becomes nearly constant. The loss of important samples may be avoided in this way.

DETERMINATION OF REDUCED COPPER.—Direct weighing of cuprous oxide is generally the most convenient method of determining reduced copper for the inexperienced chemist. This method is not "official" for plant extracts but has been shown (**6**) to give comparable results when sufficient copper can be weighed to reduce the percentage error of the fluctuations in the weight of crucibles. As a general rule, reductions giving less than 20 mg. of copper should not be weighed directly, and even at this figure, the percentage error is high. With a sufficiently large cuprous-oxide precipitate, standard methods of calculation may be used with reasonable assurance of comparable, if somewhat high, results, or an empirical copper factor may be introduced to correct for the low percentage of copper in the precipitate from plant extract reductions. This percentage has been found to be roughly constant for a given lot of material and to vary around a value of 87.2 per cent. copper instead of the theoretical value of 88.8 per cent. (**6**).

Many laboratories employ the volumetric-permanganate method of determining copper and feel that with solutions standardized and some practice in titration, it is a more rapid method than direct weighing. With the proper precautions, particularly in standardization of solutions, potassium-permanganate titration should be preferable for small quantities of cuprous oxide because it eliminates the principal source of error in direct weighing, namely, the fluctuations in the weight of crucibles. The direct titration method of SHAFFER and HARTMAN (**12**) has given irregular results with plant materials. Apparently variations in character of the tissue may affect the end-point of the titration. Under such conditions, of course, the method is worthless. The committee feels that the SHAFFER and HARTMAN method should not be recommended for general use on plant material without further trial.

The picric-acid method of estimating reducing substances has been advocated for rapid work and for the estimation of minute quantities of sugars. As modified by WILLAMAN and DAVISON (**13**), only 1 ml. of dilute sugar solution is required for each determination. This is of especial advantage when limited quantities of tissue are available for analysis.

HYDROLYSIS OF DI- AND TRISACCHARIDES.—Sucrose is the disaccharide for which this step is usually intended and the increase in reduction following inversion is frequently reported as sucrose. Maltose, raffinose, melibiose

and various glucosides, if present in the solution, will also show increased reducing power after hydrolysis with acid. Proper killing methods can usually be depended upon to eliminate maltose from consideration. This is fortunate, since maltose cannot be hydrolyzed in the presence of fructose without destroying the latter sugar. Seedlings of starchy seeds are an exception to this rule and may contain several per cent. of maltose, but they apparently contain little free fructose. Raffinose and melibiose are of limited occurrence, but may be estimated by the official method for determining sucrose and raffinose in beet products [p. 187, revised methods A. O. A. C. (**1**)]. The presence of glucosides may introduce an appreciable error in the total sugar determination unless invertase is used to invert sucrose. Acid hydrolysis under HERZFELD'S conditions [pp. 186–187, revised methods A. O. A. C. (**1**)], either 10 min. at 70° or 24 hours at 20° C., would seem to be applicable to alcohol preserved samples in the absence of glucosides. Unfortunately, glucosides are very widely distributed, and the justification of the use of acid on a particular material must rest upon the investigator. Commercial preparations of invertase are now available and detailed directions for their use are given in the revised methods of the A. O. A. C. (pp. 183–6).

GLUCOSIDES.—The glucosides as a class have received very little attention, although the work of HARVEY (**5**) on apple twigs indicates that in this instance, at least, the proportions of a glucoside seem to follow the total metabolism curve very closely. If glucosides accumulate as a result of metabolic activities, their increase should be a better measure of such activities than the disappearance of sugars, because it would be less directly affected by photosynthesis and hydrolysis.

The term glucosides includes a large number of compounds, some of which are little related beyond the fact that they are all combinations of a reducing sugar and some aromatic substance. They may react as weak bases (solanin only), or as weak acids, or they may be neutral compounds. In general they are soluble in water or dilute alcohol but not in absolute alcohol and are precipitated by basic lead acetate; the acid glucosides are also precipitated by neutral lead acetate, and all are hydrolyzed to reducing sugars and aromatic compounds by mineral acids or appropriate enzymes. The method of estimating a glucoside must, therefore, be specific as to both the glucoside to be estimated and the material from which it is isolated.

The method devised by HARVEY (**5**) for the estimation of phloridzin in apple wood is based upon the reducing value of the glucose liberated upon the hydrolysis of this compound. A portion of the solution in which sucrose has been inverted by invertase should be used. The difference in reducing value before and after hydrolyzing with 2 per cent. hydrochloric acid for fifteen minutes is calculated as glucose, multiplied by the factor 2.42 and

expressed as phloridzin. A similar method could probably be used for arbutin in pear wood. In the case of the saponins, which reduce Fehling's solution both before and after hydrolysis, some means of separation is required which is adapted to the particular compound being determined. The glucosides are of special interest, first because they may be erroneously reported as non-reducing sugars, and second because they offer a suggestive field for future physiological work.

### Carbohydrates soluble in water but not in strong alcohol

The compounds in this group are present in the plant largely as colloids which are easily suspended in water, but are precipitated by the 80 per cent. alcohol used for extracting sugars. Some of them are removed quantitatively and may be estimated from this fraction. Others are only partially soluble and should be considered here only as impurities in the soluble, or as losses from the insoluble, fraction.

DEXTRIN.—Dextrin is generally present in small quantities in starch-bearing tissue. At certain stages, dextrin may form an important part of the weight of starch storage organs. DAVIS and SAWYER (3) report as much as 4 or 5 per cent. of dextrin, on a dry weight basis, from leaves of the Irish potato, collected just after midday. Ten per cent. alcohol is frequently used for separating dextrin from starch in the residue from the alcoholic extract. When 50 per cent. alcohol is used for the first extraction, the dextrin is recovered from the extract by making it to 80 per cent. and allowing to stand. Dextrin is not easily precipitated by basic lead acetate and this material is recommended for clearing the water extract when dextrin and inulin are to be determined separately from the same sample. Neutral lead acetate is preferable at other times. The cleared extract is deleaded, hydrolyzed for 2.5 hours at the temperature of boiling water with 2 per cent. hydrochloric acid, neutralized, and its dextrose equivalent determined by copper reduction.

INULIN.—This is a condensation product of fructose which is found in many Compositae and monocots sometimes associated with starch and dextrin, although usually occurring alone. Inulin is readily suspended in water, but not in dilute alcohol. An extract containing inulin should be cleared with neutral lead acetate unless it is desired to remove this compound in order to make a separate determination of dextrin. Inulin is hydrolyzed with weak hydrochloric acid and determined as fructose. Because of the nature of the product of hydrolysis, very careful heating is required. The Bureau of Standards workers employ 1 per cent. acid and heat at 70° C. for 35 minutes.

PECTIC BODIES.—Because of their partial solubility, pectic bodies may cause difficulty in water or dilute alcohol extractions. Some of these sub-

stances precipitate as a gel upon cooling and so are not truly soluble. For this reason their determination will be considered in another section; at the same time they may seriously interfere with the handling of the water extract, particularly from fruits, fleshy stems and roots, and may even make a water or weak alcohol extraction impossible. Difficulties may be minimized by extracting with water or 10 per cent. alcohol at room temperature and by clearing this extract with neutral lead acetate before hydrolysis.

GUMS, HEMICELLULOSES, ETC.—Many materials of a gummy nature are partially soluble in water and must be removed from the dextrin-inulin fraction. Neutral lead acetate is suggested for this purpose. This solubility may also affect the total acid hydrolizable fraction. If, however, the water extract is filtered after cooling, the precipitate can be included in the determination of insoluble carbohydrates according to the outline given in another section of this report.

This report was organized by W. E. LOOMIS for the Committee.

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## THE DETERMINATION OF NITROGEN IN RELATIVELY SIMPLE COMPOUNDS*

It is assumed that the forms of nitrogen to be determined are present in the aqueous extract of plant material, which has been freed from protein, or in the solution obtained by extracting with 80 per cent. alcohol. The total amount of nitrogen in such extracts is usually rather small, and it is present in several different compounds. In no case has a complete determination of the distribution of nitrogen been made. One of the most thorough studies of this kind is that of Vickery and his co-workers on alfalfa (**10**). Because of the uncertainty as to the individual compounds present, and the practical impossibility of determining them in routine analysis, it has become customary to estimate the amounts of nitrogen present in certain forms of combination.

The determinations made most frequently are: Total nitrogen, including nitrates if these are present; ammonia nitrogen; amide nitrogen; amino-acid nitrogen; and nitrate nitrogen. Less frequently, basic nitrogen and the nitrogen of proteoses are determined. Often the sum of the various forms determined is subtracted from the total nitrogen and the difference called "Rest," "Residual" or "Other" nitrogen. The amount of this fraction and its relation to plant behavior indicate the need of its further separation and study, but methods are not as yet available for this purpose. How many of the possible determinations are to be made in any given case will depend on the amount of material available and the purpose of the study. No methods can be described which are equally useful for all plant materials. In each case preliminary studies must be made to learn the applicability of the methods and the size of aliquots best suited for use in the various determinations.

In most of the determinations the nitrogen is converted into ammonia and estimated titrimetrically. The amounts to be determined are usually small. It is necessary therefore to use especial care in selecting reagents free from nitrogen, in avoiding contamination and in carrying through suitable blanks. Solutions of $H_2SO_4$ and of NaOH of 0.02N strength will be found convenient. Each cc. of such solutions is equivalent to 0.28 mg. of nitrogen. The alkali used should be free from carbonates, and both solutions should be made with $CO_2$ free water, and preserved in well seasoned bottles. They are conveniently connected by siphons to burettes

* Section IV of the report of the Committee on Methods of Chemical Analysis of the American Society of Plant Physiologists.

with three-way stop cocks, and the NaOH solution should be protected by a soda lime guard tube. The strength of the solution should be checked at frequent intervals, as even in well seasoned bottles, the alkali is likely to increase slowly in strength.

### Total nitrogen

If nitrates may be present in the material a test should be made for them. The test should be carried out carefully and approximately quantitatively, so that in case only a trace is present a fair estimate can be made of its amount. A portion of the aqueous extract (if an alcoholic extract is used the alcohol is removed by evaporation and the residue taken up with water) is treated with a slight excess of saturated neutral lead acetate solution. The resulting mixture is then made just alkaline to litmus with NaOH. The heavy precipitate is filtered off. With most materials this treatment removes so much of the organic matter that the diphenylamine test for nitrate as described by WITHERS and RAY (**11**) can be used satisfactorily. Should the test be negative, it is advisable to repeat it on portions of the cleared solution to which enough nitrate has been added to equal one part per 100,000 and one part per 1,000,000. The test should detect one part per 1,000,000 of nitrate nitrogen in the presence of some organic matter. In pure solution it is much more sensitive than this.

A. IN THE ABSENCE OF NITRATES.—Any of the official modifications of the Kjeldahl method may be used (**1**). The determination of small amounts of nitrogen, 2 to 10 mg., and the consequent use of 0.02N solutions of acid and base, make rather extreme care necessary. The distillation may be made through block tin condensers, preferably using distilling bulbs and tubes of hard glass. It is desirable to have a very efficient trap of the Davisson or similar type in the distilling bulb. Methyl red is very satisfactory as an indicator, and the end point is sharpened materially if the distillate is boiled and cooled before it is titrated.

B. IN THE PRESENCE OF NITRATES.—The nitrogen of nitrates is fixed by combination with an aromatic compound, such as salicylic acid. The nitro groups are reduced and the total nitrogen is then liberated as ammonia by the usual Kjeldahl digestion. This method gave very erratic results until it was found that it is satisfactory on dry samples but very unreliable if water is present.

RANKER (**6**) has made a study of this determination, and the method described below is taken largely from his work.

*Reagents needed.*—Concentrated $H_2SO_4$, nitrogen free, 30 cc. of which contain 1 gm. of salicylic acid.
Sodium thiosulfate crystals.
Concentrated NaOH, 50 per cent. by volume.

*The Determination.*—A suitable aliquot of the extract is pipetted into a Kjeldahl digestion flask. It is brought to exact neutrality and evaporated *just* to dryness on a steam bath. Thirty cc. of the sulfuric-salicylic acid mixture are added and mixed thoroughly. The flask is stoppered and allowed to stand at least an hour, preferably over night. Five gm. sodium thiosulfate crystals are added and the mixture is heated for five minutes over a low flame. After cooling, 7–10 gm. of $K_2SO_4$ or anhydrous $Na_2SO_4$, and a pinch of $CuSO_4$ are added, and digestion and distillation are carried out as in the usual Kjeldahl procedure except that no $Na_2S$ is necessary, and the digest is neutralized by adding 100 cc. of the 50 per cent. NaOH.

### Amino nitrogen

A portion of the extract is evaporated on the water bath to remove alcohol. When the volume has become small, about 25 cc. of water may be added and the evaporation continued. This is repeated until all traces of alcohol have been removed. The material is transferred with water to a volumetric flask and diluted to volume. Such a volume of the original extract should be taken for evaporation, that, of the final volume, 4 cc. contains at least 1 mg. of amino nitrogen.

Amino nitrogen is determined by means of the VAN SLYKE (**9**) amino apparatus. Descriptions of the apparatus and the technique of its use, and the tables necessary for calculating the results are to be found in the standard texts of Physiological Chemistry, such as those of HAWK and BERGEIM and of MATHEWS. The micro size of apparatus can be used, with a 2 or 4 cc. sample. It may be desirable occasionally to use the larger reaction chamber with the micro-burette, so that a larger sample, up to 10 cc., may be taken.

If the solution is nearly colorless, and no great amount of buffer is present, amino nitrogen may be determined by the SØRENSEN formol titration method as described by JODIDI (**4**). Neutral formalin is added to the neutralized solution. Acidity develops due to the destruction of the base forming properties of the amino groups by the formaldehyde. The amount of nitrogen originally present in such groups is measured by the amount of base required to bring the acid solution to neutrality again.

### Ammonia nitrogen

It is rarely the case that ammonia nitrogen is present in plant extracts in amounts sufficient to make a satisfactory estimation possible. The determination must be made, however, to serve as a blank in the determination of amide nitrogen. The ammonia is aerated or distilled from a slightly alkaline solution into a measured excess of standard acid and its amount determined by titration. Any volatile bases other than ammonia that may

be present are included by this method. The amount of such bases is usually very small and their inclusion is not objectionable if the result is to be used only as a blank for the amide determination. If a more exact estimate of ammonia as such is desired it may de determined colorimetrically in the distillate by the use of NESSLER'S reagent. So far as results have been reported, however, this method has not proven entirely satisfactory with plant materials. Further studies of the use of this method are needed. For rendering the solution slightly alkaline before distillation, a slight excess of magnesia cream may be used. At the boiling temperature even this mild reagent may liberate some amide nitrogen or other nitrogen easily split off, and thus make the results for ammonia too high. The distillation is better carried out at 40° to 50° C. in vacuum. Distillation in vacuum with alcohol and $Ca(OH)_2$ is an excellent method described by VAN SLYKE (**8**) for removing ammonia from protein hydrolysates.

### Amide nitrogen

This is freed as ammonia by boiling an aliquot of the solution under a reflux for 2.5 hours with 6 per cent. HCl. The acid is neutralized and the ammonia distilled and determined as described in the previous paragraph.

The amount of amide nitrogen is found by subtracting from the total amount obtained, the ammonia nitrogen from an aliquot of the same size.

The following semi-micro methods may be used on portions of the extract freed from alcohol for the amino nitrogen determination, provided it has been concentrated sufficiently so that 10 cc. contain at least 1 mg. of amide nitrogen.

*Ammonia nitrogen.*—Ten cc. of the concentrated extract are pipetted into a pyrex test tube 30 mm. x 200 mm. The tube is connected in the VAN SLYKE-CULLEN urea apparatus with a similar receiving tube containing a measured volume, 10–25 cc., of 0.02N $H_2SO_4$. Six or seven drops of capryl alcohol (technical) are added to each tube to prevent foaming, and 15 cc. 52 per cent. $K_2CO_3$ to the sample. VAN SLYKE recommended aeration for 30 min. at such a rate that a total of at least 120 liters of air are used. As it is sometimes inconvenient to measure the rate of aeration it is safer to aerate at a rapid rate for at least 45 minutes. The excess acid may be titrated in the test tube, using the aeration tube as a stirring rod.

*Amide nitrogen.*—Another 10 cc. portion of the concentrated extract is pipetted into a pyrex test tube 30 x 200 mm. Two or three pieces of granulated pumice the size of a grain of wheat or smaller and 0.6 cc. conc. $H_2SO_4$ are added. The tube is heated to gentle boiling under a reflux condenser for 2.5 hours. This may be done easily by the low flame of a Bunsen burner if the tube rests on an asbestos board with a hole in it about one-

half the size of the tube. The solution is cooled and nearly neutralized with 40 per cent. NaOH, about 2.2 cc. being required. The ammonia is aerated as described under ammonia nitrogen except that 20 cc. of 52 per cent. $K_2CO_3$ are used. From the nitrogen found, that estimated as ammonia nitrogen is subtracted. The remainder is amide nitrogen. In both these aerations a trap tube containing $H_2SO_4$ (0.5 N is sufficient) should be inserted in order to remove ammonia from the air.

### Nitrate nitrogen

The colorimetric determination of nitrate nitrogen in plant extracts presents unusual difficulties. It is necessary (1) to remove chlorides; (2) to remove organic compounds as completely as possible by clearing; (3) to destroy such organic compounds as cannot be removed by clearing. A method using the phenol-disulphonic acid reagent has been proposed by BURRELL and PHILLIPS (**2**). This method is admittedly cumbersome, and is troublesome, too, because of the extreme care which must be used in obtaining reagents free from nitrogen. Properly carried out it is capable of yielding excellent results. It is to be hoped that further study may make this method somewhat more convenient.

Nitrate nitrogen may be reduced to ammonia nitrogen by the use of DEVARDA'S alloy in alkaline solution. STROWD (**7**) has based a method for determining nitrates in plants on this fact. Two equal aliquots of the solution, made equally alkaline are distilled, one with, the other without the addition of DEVARDA'S alloy. The ammonia obtained in the former distillate in excess of that in the latter is taken to represent the nitrogen originally present as nitrate and nitrite. CHIBNALL (**3**) has used a similar method. BURRELL and PHILLIPS (**2**) have found a loss of nitrate nitrogen by this method in the presence of ammonia and amide nitrogen.

This loss may be avoided by determining nitrate nitrogen in the solution remaining in the test tube of the VAN SLYKE-CULLEN apparatus after the removal of amide nitrogen. The contents of the tube are washed into a Kjeldahl flask to a volume of about 125 cc. and boiled down to a small volume, 15–25 cc., but not to dryness. The residue is cooled, diluted to 300 cc., and a few drops of paraffine oil are added to prevent foaming. Finally 1 gm. DEVARDA'S alloy is added and the mixture is distilled at once through a Kjeldahl apparatus into an excess of standard acid. The $K_2CO_3$ present provides alkalinity suitable for the action of the alloy, the reduction is complete, and the ammonia recovered is a measure of the nitrate and nitrite nitrogen originally present. In case this method is to be used the amide hydrolysis must be carried out with $H_2SO_4$, as some nitrate nitrogen is lost on boiling with HCl. At the beginning of the distillation the mixture

should be watched carefully, as the $K_2CO_3$ solution tends to foam badly just as it starts to boil in spite of the addition of paraffine oil. It may be necessary to remove the flame for a short time at this point. Once the boiling has well started, excessive foaming stops. This method has been used successfully in several cases, but doubtless its general applicability should be studied more thoroughly.

### Humin nitrogen

During the acid hydrolysis for amide nitrogen humin nitrogen may be precipitated. Its amount may be determined by filtering the residue from the distillation of amide nitrogen with magnesia or lime, washing and estimating total nitrogen in the insoluble residue by the Kjeldahl method.

### Basic nitrogen

The addition of phosphotungstic acid to the acidified filtrate from the humin determination results in the precipitation of basic nitrogen. The HAUSMANN method, as modified by OSBORNE and HARRIS (**5**) for the determination of basic nitrogen in protein hydrolysates may be adapted for this estimation.

### Proteose nitrogen

CHIBNALL (**3**) precipitates protéoses from protein-free plant extracts by saturating the acidified solution with zinc sulphate. The precipitate is washed, redissolved, reprecipitated, washed again, redissolved and made up to a definite volume. Total nitrogen is determined in an aliquot of this solution by the Kjeldahl method.

This report was organized by T. G. PHILLIPS for the Committee.

C. O. APPLEMAN,
W. E. LOOMIS,
T. G. PHILLIPS,
W. E. TOTTINGHAM (chairman),
J. J. WILLAMAN.

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# BRIEF PAPERS

## AN APPARATUS FOR CONTROLLING THE FLOW OF NUTRIENT SOLUTIONS IN PLANT CULTURES

(WITH ONE FIGURE)

Each advance made in plant nutritional studies brings with it the realization that ultimately some satisfactory method of experimentation is yet to be devised whereby low concentrations of a given salt solution may be maintained in contact with the roots of plants. A solution of this problem may be approached by continuously renewing the culture medium. Several investigators[1] have already applied this method and described their apparatus. One phase of an investigation dealing with low potassium requirements of the tomato plant led to the devising of an apparatus of large capacity and flexible enough to be adapted to various types of experiments involving flowing solutions. All essential parts can be made and put together from materials usually found in plant physiological and chemical laboratories.

The two essential units of the apparatus are represented in the accompanying figure on tables *T* and *T'*. The plant container, *P*, is a 5-gallon glazed, earthenware jar. A special cover made of heavy sheet copper fits each of the 24 jars used in these experiments. Five holes were cut in each cover and then a heavy coating of tin was applied as a precaution against copper poisoning. These holes were large enough to receive the flat cork stoppers commonly used to support plants in ordinary fruit jars. The rate of flow from the plant container into the waste tube, *F*, is controlled by raising or lowering the siphon, *E*, which fits tightly into one of the cork stoppers of the cover. A delicate adjustment of stop-cocks is thus entirely eliminated. The rate of flow in one experiment was adjusted to approximately 7 liters per day per each jar, which in this case contained a single plant. Higher and lower rates can easily be had by simply adjusting the height of the outlet siphon.

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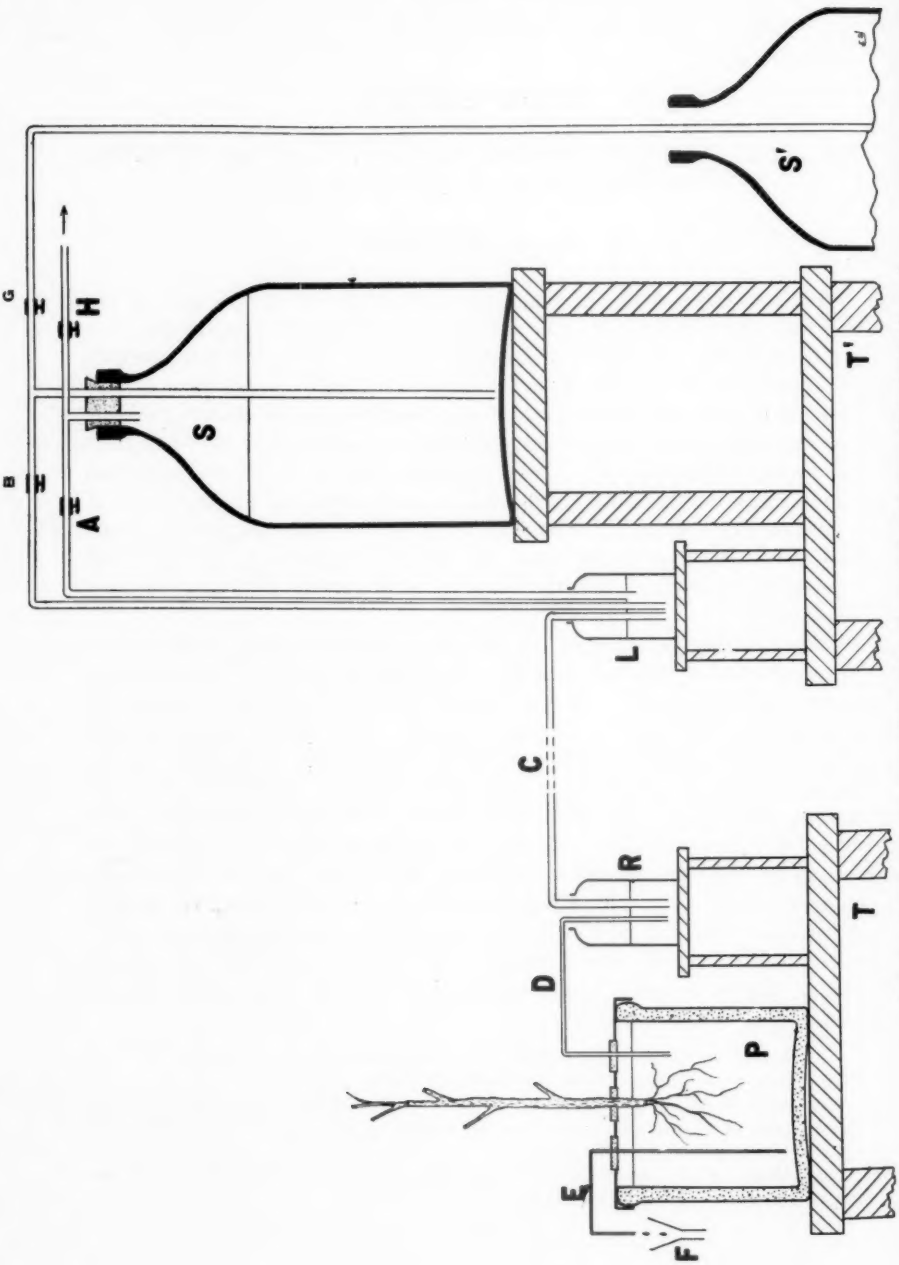


FIG. 1. Apparatus for controlling flow of nutrient solutions. Description in text.

In the experiment just noted, six 5-gallon jars were connected to a sub-reservoir, *R*, by means of siphons, *D*. As illustrated in the figure, this reservoir is connected by siphon, *C*, to the other unit of the apparatus, the constant level jar, *L*. A desired level is maintained by a modification of the well-known Mariotte flask. When a solution in *L* drops below the end of tube *A*, air enters the 10-gallon bottle, *S*, and with its partial vacuum thus destroyed fresh solution flows into *L* through the siphon *B* until the solution in *L* again rises high enough to enter tube *A* and attain a height that balances the partial vacuum in *S*. It is of course necessary that an air tight stopper be used in bottle *S* and that the stop-cocks, *G* and *H* be closed. Tube *A* should be approximately 7 mm. inside diameter with the lower end cut or ground at an angle. This prevents water from "hanging" in the tube when air should be entering. New solution is made up in bottle *S'* and then drawn up into bottle *S* by applying suction at *H* after opening the cocks *G* and *H* and closing *A* and *B*. It is well to paint the glass tubing and bottles black. This excludes enough light to prevent the growth of algae.

Attention is called to one essential difference between the types of apparatus heretofore described and the present one. In the former cases the rate of flow of nutrient solution is regulated before the solution enters the plant container. This is accomplished either by delicately adjusting stop-cocks or by passing the solution with a given hydrostatic head through proper lengths of capillary tubing. In the apparatus here described the outlet siphon controls the rate at which the solution flows from the plant container. This rate, together with that of transpiration, determines the rate of flow into this container. While the rate of flow through the outlet siphon remains practically constant for a given adjustment, that of transpiration may vary from hour to hour as well as from day to day. There is thus a definite amount of solution flowing from the plant container per unit of time which is always in excess of the transpiration by an amount depending on the adjustment of the outlet siphon.

It is a pleasure to acknowledge indebtedness to Professor D. R. Hoagland, of the Division of Plant Nutrition, University of California, for the facilities and equipment used in conducting the experiments involving the apparatus herein described.—Earl S. Johnston, *Laboratory of Plant Physiology, University of Maryland.*

Fig. 1. Apparatus for controlling flow of nutrient solutions. Description in text.

## DEVICES FOR SOWING AND GROWING SPORES

(WITH TWO FIGURES)

The usual but somewhat antiquated methods for sowing spores and small seeds in laboratory cultures results in great unevenness in distribution. In many places on the culture media the spores fall in masses and are then too dense for even growth, while other areas may be missed entirely. Despite much good work that has been done by these old methods, especially with certain spores, experience has shown that more uniform sowing of spores and seeds conduces to more normal growth and behavior of the structures that the spores produce. Prothallia and protonemata are capable of growing very densely and often do so in artificial cultures here referred to. Their ability to grow very densely exceeds that of most of the higher plants. However, such compact growths as are often seen when grown artificially are rarely, if ever, seen in nature owing to proper dissemination. Although it is clear that excessively dense growth in this respect is disadvantageous, a critical comparative study as regards the degree of influence exerted by growth density has yet to be made.

The writer, however, has constructed two devices which make it possible to sow spores very uniformly and to grow them in a better way than is possible by the usual methods.

The first of these, which is for sowing the spores, is shown in figure 1. It consists of a clear glass cylinder A, 5 cm. in length and has a uniform diameter of 16 mm. This size has been found convenient, although larger or smaller sizes of these glass cylinders are sometimes advisable, according to the volume of material available. A much smaller cylinder is preferable where only a small quantity of spores can be obtained. A cork B, one cm. in length and that just fits the cylinder closes the latter when not in use. Through the center of the cork is passed a glass tube C, having a bore of 4 mm., a length of 11 cm. and flared at its upper end. A cylinder of Swiss silk gritgauze D, 9 cm. long and that fits both cylinder and cork closely, is held to each of these by means of tight rubber bands, E. Such a cylinder of new gritgauze is stiff enough, if properly made, to support the weight of the glass cylinder when in a horizontal position and will not collapse easily. It is therefore more suitable than any other material, except metal gauze which, of course, should not be used for this purpose. The flared tube C is used to introduce spores into the sterilized glass cylinder without opening the latter. The tube is then plugged with cotton. It also assists

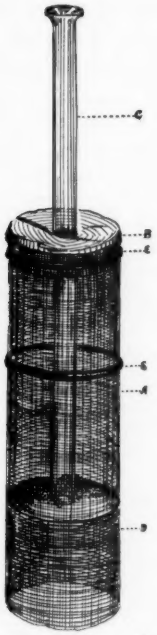


FIG. 1. Device for sowing spores.

in revolving the contrivance while sowing the spores and enables one to adjust the position of the glass spore container in the gritgauze cylinder D. A gritgauze cylinder having 4 to 6 meshes to the mm. sows fern or moss spores thickly enough if properly manipulated. For certain small seeds gritgauze having meshes 2 mm. square may be obtained. Figure 1 shows the apparatus closed, when it serves as a sterilized container. By shoving the glass tube C through the cork B, the desired adjustment of the glass spore container in the gritgauze cylinder is attained. In this way narrow or wide swaths of spores may be sown. When the apparatus is extended the spores are allowed to slide to the cork. The cylinder is then rotated in a horizontal position which allows the spores to be sown evenly and to the desired density. This should first be tested by passing the rotating cylinder over white paper. By means of this test it can be seen that great uniformity as to distribution is obtained. By holding the apparatus close to the sterilized culture medium while operating, the drifting of the spores by currents of air is avoided which is hardly possible by former methods. It is further advisable to place the culture inside of a sterilized glass case and then sow the spores.

The second device is for growing the spores as is shown in figure 2. It consists of a circular zinc box F, 25 cm. in diameter and 6 cm. deep. In the roof of this box is a circular opening G, the rim of which, as well as the periphery of the box, is turned up at right angles to a height of 12 mm. in order to hold a layer of distilled water for moisture control. The sides of the box F, are covered for part of their height by a strip of brass wire

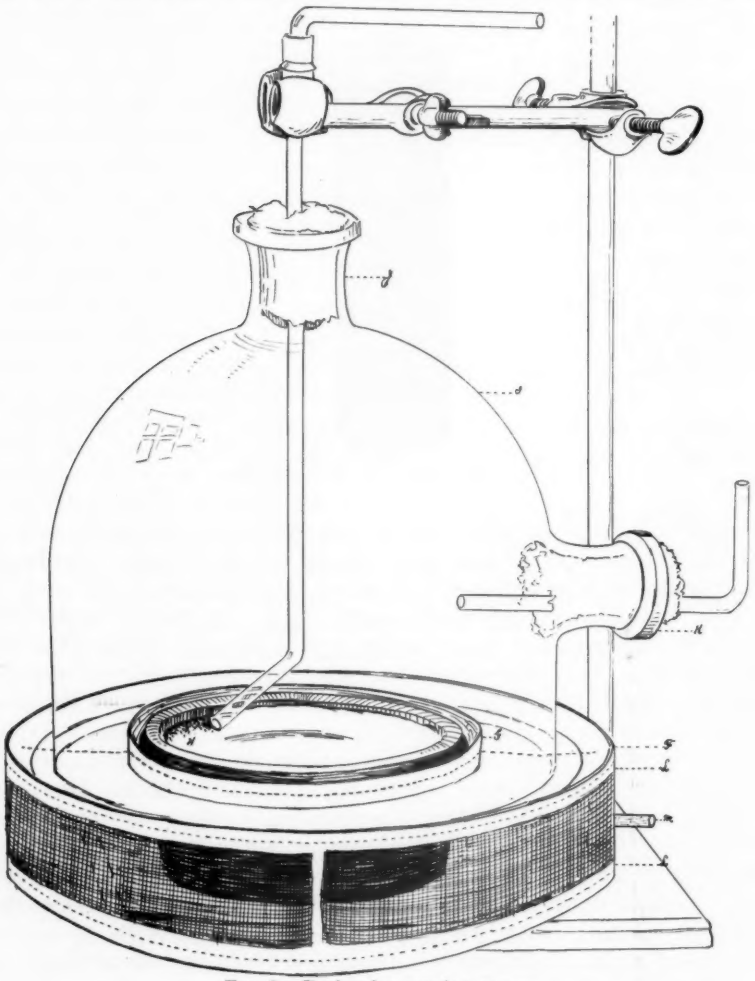

FIG. 2. Device for growing spores.

gauze having 8 meshes to the mm. and in addition this may be supplemented by a sterilized cloth covering, where necessary. The culture of spores H, sown as above indicated, is placed flush with the top of the central opening G. Over this is placed a low bell-jar I, with its lower edge submerged in the water. The shorter the bell-jar the better, but any tall bell-glass may be quickly cut off to the desired height by means of a number 20 nichrome wire carrying a 110 or 220 volt electric current which may be regulated by a rheostat. A terminal tubulure J, or better a lateral one K, carries a glass tube held in place by cotton or a rubber stopper according to requirements. This tube may be used for watering the culture without opening the apparatus, or for connecting with an aerating contrivance for the passage of air. If temperature control is necessary, a thermometer may be enclosed in I, and any desired temperature maintained. When carefully put together this piece of apparatus excludes the fungus-gnat and other insects, and obviates the danger of contamination, especially by moulds which often partly or completely destroy such cultures when grown by less controlled methods.

The fine brass wire gauze on the sides of F may be closed for certain experiments by a special contrivance. This consists of an adjustable metal draw-band L, wide enough to cover the brass wire gauze and provided with a tube M. It is coated with paraffin and made gas tight by means of plastilina or a suitable gasket on which the band is drawn tight. The interior of F should also be heavily coated with paraffin of a high melting point in order to insulate the surface of the metal, and a layer of paraffin oil substituted for the distilled water at G. For certain studies the bell-jar I may be cemented gas tight to the roof of F by a stiff air pump wax-mixture. The arrangement just described, especially when the spores are grown on a suitable substratum, extends the usefulness of the apparatus by enabling the operator to perform a great variety of experiments of physical or chemical nature directly on the spores themselves, or on the structures they produce during germination or during normal growth.—F. M. ANDREWS, *Indiana University.*

## IONIZATION AND ADSORPTION ISOELECTRIC POINTS

In considering the isoelectric points of membranes it is evident that these points may be produced in two manners. First, there is the commonly known isoelectric point produced by the ionization of ampholytes. Second, the membrane without going into solution appreciably may show an isoelectric point produced by differential adsorption. This fact of adsorption producing an isoelectric point has been largely overlooked in the discussion of the isoelectric point of membranes. F. E. BARTELL in his discussion on "Anomalous Osmose" in the First Colloid Symposium Monograph makes no definite statement of this although his graphs show that it exists. On calling this point to his attention I received a reply which I believe will be of interest to physiologists, which I quote here.

"In connection with solid material as particles or as membranes which appear to exhibit an electrical potential against water, I have assumed that an iso-electrical point is possible. This idea, I believe, has been carried along since the time of PERRIN'S work. For example, in W. W. TAYLOR'S "The Chemistry of Colloids," p. 69, referring to PERRIN'S work, he states: "Negative diaphragms become more negative in alkaline solutions; in acid solutions the diaphragms become less negative, and with increasing concentration of acid they become electrically neutral and finally positive."

"I have regarded this electrically neutral point as a definite iso-electric point. In my past work I have accepted the HELMHOLTZ idea of the electrical double layer and have assumed that this layer ($\varepsilon$) in aqueous solutions is largely the result of adsorption. By making use of this assumption many of the phenomena obtained with membranes appear to be fairly simple. As you are aware, FREUNDLICH has recently pointed out that the electrical double layer conception of HELMHOLTZ is not sufficient, that we must consider an electro-kinetic potential ($\zeta$), which is not the same as the other. In case this latter view is correct, we can make use of the electrokinetic potential and carry out the general treatment as before. Considerable work is now being done along this line by different investigators and undoubtedly we will have some worthwhile contributions appearing in the near future."

Will it make for better clearness to refer to these conditions as *ionization isoelectric points and adsorption isoelectric points?*—R. B. HARVEY, *University of Minnesota.*

## NOTES

**First International Congress of Soil Science.**—In 1924 the Fourth International Conference of Soil Science held at Rome, resulted in the organization of an International Association of Soil Science. The first international congress of this Association wil be held in Washington, D. C., under the presidency of Dr. J. G. LIPMAN, of Rutgers University, June 13–22, 1927. Four of the six International Commissions making up the Association deal with problems of vital interest to plant physiologists. These are the Commissions on Soil Physics, Soil Chemistry, Soil Bacteriology, and Soil Fertility. Arrangements have been made to present to the readers of PLANT PHYSIOLOGY an account of such features of the Congress as may be of interest to physiologists.

**Fifth National Colloid Symposium.**—The preliminary program of the fifth National Colloid Symposium, to be held at Ann Arbor, June 22–24, 1927, shows a very attractive program. Several of the papers should prove very valuable to students of plant physiology. Dr. GORTNER and his coworkers will discuss the proteins and the lyotropic series, MICHAELIS reports his investigations on molecular sieve membranes, and STAMM discusses electroendosmose through wood membranes. Many other papers deal with the influence of electrolytes, surface phenomena, adsorption, etc., topics helpful in bringing new interpretations to life processes in plants and animals. There is a special symposium dealing with plasticity and its measurement. These colloid symposia are very helpful, and plant physiologists are welcome to the privileges of the meeting.

**Back Numbers.**—Attention is called again to the diminishing supply of complete sets of PLANT PHYSIOLOGY. The limit on the number of complete sets is set by the January number of 1926. Some of these first volumes are going to foreign subscribers, and every set sold deprives some American plant physiologist of the opportunity to own a complete file. The number remaining unsold would supply only a fraction of the demand if every physiologist desired to own a complete set of the journal.

**Manuscripts.**—The publication of PLANT PHYSIOLOGY is delayed each quarter because manuscripts are not received in sufficient number to catch up with the publication date. At the time of going to press with each number, the editors are using practically all the material on hand. Mem-

bers of the American Society of Plant Physiologists are invited to submit their best work to the journal, and to encourage others to use PLANT PHYSIOLOGY as a medium of publication of the best research. In order to catch up with the calendar we need a sufficient number of papers to make at least one additional number of 100 to 150 pages. Some of the delay can be avoided if authors will always be prompt in correcting and returning galley proofs. Those who have worth while papers rounding into shape for publication are invited to send them to some member of the editorial committee. All papers accepted will be given prompt publication, as long as the supply of papers does not exceed the funds available for their publication.

**Notes.**—The members of the American Society of Plant Physiologists are invited to submit brief notes concerning events of general interest, to be used in connection with this section of PLANT PHYSIOLOGY. Information regarding meetings of various kinds should be sent in several months before the meetings are held, if possible. Any other information that possesses general interest may be submitted to the editors for use in this section of the journal. These notes may be made a very helpful feature, if the members of the Society help to increase their usefulness.

**The Purdue University Section.**—The Purdue University Section is a very active group. Arrangements have been made to allow students to become associated with the Section and enjoy the privileges of the meetings as associate members. This arrangement should be stimulating both to the members of the Section and to the students associated with them in the meetings. The local vice president is Prof. J. F. TROST. The meetings of the Section are delightfully informal, and the results of the meetings show that local sections are valuable to the institutions where they have been organized.

**The Minnesota Section.**—The local Section of the American Society of Plant Physiologists at Minnesota has made an enviable record during the current year. According to Prof. A. C. ARNY, local vice president of the Section, there are now 21 members of the Society in the Minnesota Section. That is a splendid record, and should stimulate other institutions to develop sections of the Society. The Section holds an occasional luncheon, and recently had the privilege of attending a lecture by Dr. W. J. V. OSTERHOUT in the Chemistry Auditorium. Conditions at the University of Minnesota are favorable to the development and maintenance of a virile and enthusiastic Section.

**Soil Conditions and Plant Growth.**—The fifth edition of this splendid monograph in the Rothamsted series on Agricultural Science has been published recently by RUSSELL. All of the chapters except the historical introduction have been rewritten, and the book enlarged about 25 per cent. in number of pages. Some changes occur in chapter headings, and one change in order of presentation. Thus, chapter IV, formerly Colloidal Properties of the Soil, is now entitled Chemical and Physical Relationships of the Soil. Chapter VII has become Chapter VI in the new edition. Other minor changes in chapter headings are noted. The book has been enormously useful, and the fifth edition will be welcomed as the best treatise on the subject available in English. The book comes from the press of Longmans, Green and Co., price $6.50. It deserves to be in a more substantial binding.

**Enzymes.**—Those interested in enzymes, their properties, distribution, methods of preparation and study, and practical applications of enzyme activity, will find this book by S. A. WAKSMAN and W. C. DAVISON full of interest. It contains fifteen chapters, of which chapter VI deals with plant enzymes, and chapter VII with the enzymes of micro-organisms. Chapters X, XII, XIII, and XIV also have much for the plant physiologist, since they deal with the enzymes acting on carbohydrates, oxidizing and reducing enzymes, zymases, and catalase. The book is simply entitled Enzymes, and is published at $5.50, by Williams and Wilkins, Baltimore, Maryland.

**Principles of Soil Microbiology.**—This book by SELMAN A. WAKSMAN, of Rutgers University, is a monumental piece of work, which presents an exhaustive survey of the field of soil microbiology. The book is divided into four sections; the first, of only one chapter, considers the occurrence and differentiation of micro-organisms in the soil. The second section, on isolation, identification and cultivation of soil micro-organisms, contains thirteen chapters each dealing with some different type of organism, ending with algae, fungi, actinomyces, protozoa, and non-protozoan fauna. The third section takes up the chemical activities of micro-organisms, their metabolism, energy transformations, decompositions of non-nitrogenous and nitrogenous material, oxidation and reduction processes, nitrogen fixation, and sulphur transformations by soil organisms. There are nine chapters in this section. The final section, soil microbiological processes and soil fertility, also contains nine chapters dealing with various phases of bacterial and other biological processes as related to the problems of plant nutrition. The final chapter in this section is a very brief orientation chapter on the history of soil microbiology, its past, present, and future. Such a book will be exceedingly useful to physiologists. The price is $10.00, but the

book contains nearly 900 pages. The Williams and Wilkins Co. are the publishers.

**New Conceptions in Colloidal Chemistry.**—Under this title, E. P. Dutton and Co. announce the publication of a new book by HERBERT FREUNDLICH. The book contains nine chapters, as follows: Adsorption; the electrokinetic potential; adsorption, valency, and coagulation; the rate of coagulation; the stability of hydrophilic soils; the state of aggregation and the shape of colloidal particles; extent and change of surface in colloidal systems; photodichroism and similar phenomena. The price is $2.00, with discounts to instructors.